Remarks

Claims 1-7, 12-14 and 23-27 are pending. Claims 1, 12 and 23-26 have been amended. Independent claim 1 is presently amended to clarify the administered composition as consisting of the combination of (1) an (a) NSAID, cytokine inhibitor or a mast cell inhibitor and (b) a MMP inhibitor or (2) an (a) NSAID, (b) cytokine inhibitor or mast cell inhibitor, and (c) MMP inhibitor. Support for the amendment to claim 1 can be found, for example, at page 4, lines 6-11 (using a combination of disclosed inhibitors); and lines 23-25 (wherein at least one of the agents is an NSAID). Also, please see page 5, lines 8-15, for further support. Claim 12 was amended to be consistent with the amendments to claim 1. Claims 23-26 were amended to provide proper antecedent basis.

Rejections Under 35 U.S.C. § 103

Claims 1, 4-6, 12-14 and 23-27 were rejected under 35 U.S.C. § 103(a) as obvious over Shenk, et al. *Clin. Oral Implants Res.* 8(5):427-433 (1997) ("Shenk"); or Rothwell, et al *Spec. Care Dentist.* 10(1):21-25 (1990) ("Rothwell"), in view of Tilg, et al *Transplantation* 56(1), 196-201 (1993) ("Tilg"). Claim 7 was rejected under 35 U.S.C. § 103(a) as obvious over Shenk *et al.*; Rothwell *et al* and Tilg *et al.* as applied to claims 1, 4-6, 12-14 and 23-27 above, and further in view of U.S. Patent No. 6,239,119 to Stogniew *et al.* Applicants respectfully traverse these rejections to the extent they are applied to the claims as amended and in view of the accompanying data showing unexpectedly better results with the combination of a MMP inhibitor (a tetracycline) and an NSAID.

The Data

As demonstrated by the enclosed report "Biomodels Final Report Studies ORA-01 through ORA-05", NSAIDs alone are not effective in treating mucositis; and an MMP inhibitor such as one of the tetracyclines is effective. However, the combination is more effective than the sum of either (since the NSAID alone is ineffective, one would expect the two in combination to be less than or equal to the value of the MMP inhibitor alone). For example, referring to the data in Table 3, it is apparent that the efficacy of minocylcine is statistically significant by day 10 at a dosage of 0.1 mg minocycline/ml. In contrast, indomethacin, an NSAID, is ineffective. See also Figures 7, 10 and 11, showing no differences between placebo and Flubiprofen, Ketorolac, and Etodolac. In contrast, see the data in Table 11 for minocycline 0.1 mg/ml and Flurbiprofen 20 mg/ml.

Schenk

Schenk describes the treatment of periodontal disease via application of topical tetracycline HCL in the treatment of perimplant mucositis (abstract).

This is **not** mucositis resulting from chemotherapy or radiation as claimed, nor is the class of patients the same – those receiving an implant for treatment of periodontal disease are different from those to be treated with chemotherapy or radiation. There is a different mechanism of action, different clinical course, and different responsiveness to drugs.

Rothwell

Rothwell discloses the treatment of mucositis by administering a combination of tetracycline, hydrocortisone, nystatin and diphenhydramine (page 22, col. 2). There is no

disclosure that one should substitute an NSAID, mast cell inhibitor or cytokine inhibitor for the steroidal antiinflammatory, antifungal and antihistamine, nor has the examiner provided the requisite motivation to do so.

Tilg

Tilg describes studies in culture *in vitro*, ***not in animals***, showing that pentoxifylline blocks the proliferative response of PBMCs to TNF-alpha (abstract). There is a statement in the abstract that pentoxifylline is useful in the treatment of bone marrow patients, reducing major complications. Presumably these studies were done using systemic administration of the drug, not topical. However, there is no information on how the drug would be given to patients, no dosage information, no data, and no description of what other drugs may have also been used. There is no data that indicates the in vitro studies would be predictive of *in vivo* results. Therefore one skilled in the art would not know how and when to use the composition.

Stogniew

Stogniew describes administering a radioprotectant topically to prevent mucositis, which may additionally include any number of other excipients (see col. 9, lines 42-63). The specific drug, amifostine, is hypothesized to protect by forming an active free thiol once inside the cell, which acts as a scavenger for oxygen free radicals (col. 2, lines 12-21). This drug is not one of the claimed components, i.e., it is not an NSAID, inflammatory cytokine inhibitor or mast cell inhibitor, nor has the examiner cited any reference that one of ordinary skill in the art would know to substitute the claimed compounds for a radioprotectant such as amifostine.

U.S.S.N. 09/800,855
Filed: March 7, 2001
AMENDMENT AND RESPONSE TO OFFICE ACTION

Summary

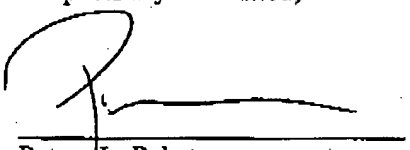
It is not seen where any of the cited documents leads one of ordinary skill in the art to the claimed combination with a reasonable expectation of success that the combination will be useful for the treatment, inhibition, or prevention of mucositis in a human patient, wherein the patient is in need of or undergoing radiation treatment or chemotherapy. The references, either singly or in combination, do not teach each and every element of the claimed method.

Claim Objections

Claims 23-26 were objected to for being dependent upon a canceled claim. Claims 23-26 have now been amended to properly depend from independent claim 1.

Allowance of claims 1-7, 10-14 and 23-27 is respectfully solicited.

Respectfully submitted,



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Biomodels

Final Report

Studies ORA-01 through ORA-05

EFFECT OF SELECTED COMPOUNDS ON EXPERIEMENTAL RADIATION-INDUCED MUCOSITIS

Successful attenuation of oral mucositis by minocycline and related compounds.

August 3, 1999

1. ABSTRACT

This report describes the results of studies examining the efficacy of compounds to treat or prevent radiation-induced, oral mucositis in hamsters. Minocycline was found to have profound activity in the attenuation of oral mucositis. Subsequent studies confirmed the effect of minocycline, identified optimum dosing conditions, and evaluated other potential compounds for use in combination with minocycline. Related compounds (doxycycline and tetracycline), showed similar activity while the non-tetracycline antibiotic, metranidizole, was ineffective.

Studies ORA-01 and ORA-02 evaluated six (6) compounds for efficacy. The selection of compounds was based on the methods and models of Dr. Sonis regarding the mechanisms of induction of oral mucositis as described in US Patent Applications U.S.S.N. 09/265,299 and U.S.S.N. 09/265,299. Five of the six compounds were ineffective or marginally effective. However, minocycline, when used at a concentration of 0.1 to 1.0 mg/ml, reduced the level of oral mucositis induced by exposure to acute radiation such that a clinical cure was achieved.

Study ORA-03 repeated the observations of ORA-01 relative to minocycline, and showed that the related compounds tetracycline and doxycycline inhibited the development of oral mucositis in hamsters, in a manner similar to that observed with minocycline. This study suggested that the tetracyclines, as a class, might act to prevent oral mucositis or hasten its resolution.

A dose-response study (ORA-04) again confirmed the original minocycline results and demonstrated that the 0.01mg/ml and 0.001 mg/ml doses were not effective in the treatment of oral mucositis in hamsters. To support the hypothesis that the effect of the tetracyclines was not dependent on their antimicrobial activity, the non-tetracycline antibiotic metronidazole was tested at 0.1 and 1.0 mg/ml doses. Metronidazole failed to demonstrate any effect on the course or severity of oral mucositis. This result indicated that the mechanism by which the tetracyclines act on oral mucositis was unlikely due to its antibiotic activity.

In a metanalysis of minocycline treatments from ORA-01, ORA-03 and ORA-04, untreated animals demonstrated clinically significant mucositis in 278 out of 576 possible days. In contrast, animals treated with minocycline at a dose of 0.1 mg/ml demonstrated clinically significant mucositis in only 34 out of 576 possible days, a reduction of 88%.

In the final study of this series (ORA-05), minocycline was used alone and in combination with flurbiprofen. A cyclodextrin vehicle was used to solubilize the flurbiprofen. When used as a single agent, minocycline at 0.1 mg/ml reduced the severity of oral mucositis as observed in the previous studies. Flurbiprofen, on the other hand, when used at 20 and 2 mg/ml doses, not only failed to reduce the severity of mucositis, but also caused a significant worsening of the condition. The combination of minocycline and flurbiprofen showed no efficacy in the treatment of oral mucositis; the addition of flurbiprofen to minocycline abrogated the beneficial effect of the former. This observation may be of special importance given equivocal effects of improvised mouthwashes that include tetracyclines as one of many components.

2. INTRODUCTION

2.1 Background

Mucositis induced by antineoplastic drugs is an important, dose-limiting and costly side effect of cancer therapy. The ulcerative lesions produced by stomatotoxic chemotherapy and radiation are painful, restrict oral intake, and cause interruptions in the treatment regimen. In granulocytopenic patients, the ulcerations that accompany mucositis are frequent portals of entry for indigenous oral bacteria often leading to sepsis or bacteremia. The overall frequency of mucositis varies and is influenced by the patient's diagnosis, age, level of oral health and the type, dose and frequency of drug or radiation administration. Some degree of mucositis occurs in approximately 40% of patients who receive chemotherapy. About one-half of those individuals develop lesions of such severity as to require modification of their cancer treatment and/or parenteral analgesia. The condition's incidence is consistently higher among patients undergoing conditioning therapy of bone marrow transplant, continuous infusion therapy for breast or colon cancer and therapy for tumors of the head in neck. Among patients in high-risk protocols, severe mucositis occurs with a frequency in excess of 60% and in virtually all patients who receive radiation for head and neck cancer. In the latter group, mucositis typically begins with cumulative exposures of 15 Gy and then worsens as total doses of 60 Gy or more are reached.

As a consequence, it is not unusual for mucositis to necessitate a de-escalation of a planned dosing regimen. Because of the concomitant neutropenia that often occurs secondarily to chemotherapy-induced myelosuppression, mucositis is a significant risk factor for systemic infection. Patients

with mucositis and neutropenia have a relative risk of septicemia that is greater than four times that of individuals without mucositis.

In addition to its impact on quality of life and morbidity and mortality, mucositis also has a significant economic cost. For example, in patients undergoing autologous bone marrow transplant for hematological malignancies, the length of hospital stay among patients with mucositis was five days longer than those without the condition. At an average day rate of $4,500 for this patient population, this results in additional charges of more than $20,000 per patient. In addition, mucositis leads to hospitalization of otherwise ambulatory patients, feeding-tube placement and increased medication use.

Clinically mucositis is characterized by four stages:

- An initial stage in which there is some slight erythema with patches of keratosis.
- An erythematous stage during which patients feel sensitivity to spicy foods and heat, somewhat analogous symptomatically to a pizza burn.
- The ulceration-pseudomembrane stage in which there are full-thickness breaks in the oral mucosa and which is accompanied by severe pain often requiring narcotic intervention.
- Spontaneous healing which occurs about two to three weeks after the cessation of antineoplastic therapy if there is no secondary infection.

The development of effective treatment for the prevention and elimination of mucositis has been elusive. The range of medications or devices that have been tried for mucositis is extensive and includes topical antimicrobials, marrow-stimulating cytokines, vitamins, inflammatory modifiers, palliative rinses, amino acid supplements, cryotherapy and laser treatment. Despite the wide range of therapeutic approaches to its treatment, none has proven to be consistently effective. It is likely that the lack of a successful intervention is at least in part, due to a failure to recognize the biological complexity in the pathogenesis of mucositis.

Mucositis as a biological process has only recently been appreciated and involves the sequential interaction between the challenging agent, th oral epith lium, connective tissue, endothelium, free radicals, cytokines, and the oral environment including the bacterial flora and saliva. Damage to endoth lial and epithelial cells and connective tissue results in the release of free radicals and a

cascade if pro-inflammatory cytokines, including tumor necr sis factor-α, Interleukin-1β and Interferon-γ. The breakdown of connective tissue fibronectin, an early occurrence in the mucositis process, further stimulates the release of these molecules. It appears that in the case of radiation, cytokine release occurs at doses of x-ray therapy that are not directly damaging to tissue. TNF is itself damaging to tissue and may be an initiating and accelerating event in the mucositis process. The ability of IL-1 to incite an inflammatory response, which results in increased submucosal vascularity, may have the potential to increase local levels of cytotoxic agent. Increasingly, data suggest that the release and/or presence of free radicals and pro-inflammatory cytokines are the seminal event in mucositis development and that successful attenuation of these molecules has a favorable impact on the development and course of the condition. Additionally, both direct and indirect effects to epithelial cells result in either apoptotic or necrotic changes of basal epithelial cells; differentiation into new epithelial cells is halted. The arrest of epithelial cell renewal leads to atrophy followed by ulceration.

Since the development of mucositis appears to be markedly dependent upon the release of free radicals, the expression of pro-inflammatory cytokines and the degeneration of endothelium and connective tissue, we reasoned that single or multi- agent therapy targeted at interrupting this sequence would be of benefit in the prevention and treatment of mucositis. In addition to its antibiotic properties, minocycline has these activities. In this regard, minocycline has been studied relative to both its potential uses in toxic shock, dermatitis and arthritis. Kloppenburg et al demonstrated that minocycline exerted an inhibitory effect on TNF-α and IFN-γ production by stimulated T-cells. This observation was confirmed and extended by Milano and colleagues who suggested that the ability of the tetracylcines to down-regulate pro-inflammatory cytokines was mediated through the ability of these agents to decrease inducible nitric oxide synthetase. Minocycline's capacity to effectively modulate proinflammatory cytokines has been confirmed by other investigators. In addition, it is possible that minocycline and related compounds might provide additional benefit in the course of mucositis from their activity as metalloproteinase inhibitors. Overall, the observation that the tetracyclines vary in their specificity for MMP inhibition suggests that this aspect of their activity is less important than those previously discussed.

The Principal Investigator has developed a hamster model of chemotherapy-induced mucositis and, more recently, a model of radiation-induced mucositis. In th latter model, specific doses of acute radiation are targeted to the designated mucosa, with protection of other areas by a customized lead

shield. The reproducibility of the model has been validated, with the consistent appearance of ulcerative mucositis between Days 15 and 18 following radiation. Using this model, the efficacies of various topical agents have been tested for their abilities to modify the course of radiation-induced mucositis.

2.2 Study Design and Objectives

Each study described here had an identical design as shown in Table 1, a flow diagram indicating the tasks and timing of events in the studies. The studies involved a 30 day protocol where randomization and dosing began on day -1. Radiation occurred on day 0 and the course of oral mucositis was followed until day 28. Peak mucositis was usually observed on days 14-16, followed by a healing phase that was resolved by day 28. Fifty-six (56) hamsters were randomly divided into seven treatment groups with eight (8) animals per group. Each group was assigned a different treatment as follows:

Group 1: No vehicle control; distilled water, was used.

Groups 2 through 7 were the experimental groups. In all experiments except ORA-05, a single agent was used at two or more different concentrations, usually representing 10-fold variations in dose. ORA-05 was a study of combinations of agents where cyclodextrin was used as a vehicle. A separate vehicle control group was included in the study.

Induction of mucositis by an irradiation regimen. In all five studies, an acute radiation dose of 35 Gy on day 0 was administered in order to produce severe mucositis around day 15. The use of acute radiation to induce mucositis was preferable to the use of either fractionated radiation or chemotherapy for these initial studies. The acute model had little systemic toxicity, resulting in fewer animal deaths. This fact permitted the use of smaller groups (N=8) in the initial studies. The acute model has been used successfully to demonstrate the presence or absence of efficacy for a large number of compounds. The acute radiation model was therefore appropriate as an initial protocol for the screening of diverse families of compounds

Dosing schedules and dose response. Dosing was done topically for all compounds. The hamsters were dosed three times per day by means of a needleless syringe containing the 0.1 ml volume of the experimental compound. The compound was injected directly into the cheek pouch of the

unanesthetized hamster. Dosing began on day –1 and continued daily until day 21. The decision to use at least two different concentrations of each compound in these experiments allowed the observation of a dose response. If one of the doses was inappropriate, either above or below the biologically effective range for efficacy, the second dose might permit the demonstration of efficacy thereby lowering the chance that a potentially good candidate might be excluded due to an inappropriate choice of dose.

Starting on day 6 and then every second day thereafter (Days 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28), all animals were photographed and evaluated for mucositis scoring as described below.

3. MATERIAL AND METHODS

3.1 Study Locations

The studies were performed at the University of Massachusetts Medical School, Worcester MA. (ORA-01, ORA-03 and ORA-05) and at the Massachusetts College of Pharmacy, Boston, MA. (ORA-02 and ORA-04). Irradiation of the animals was carried out at The University of Massachusetts Lowell, MA. (ORA-01, ORA-02 and ORA-03) and at the Dana Farber Cancer Institute, Boston, MA (ORA-04 and ORA-05).

3.2 Animals

Male Golden Syrian hamsters (Charles River Laboratories or Harlan Sprague Dawley), aged 5 to 6 weeks, with body weight approximately 90 g at study commencement, were used. Animals were individually numbered using an ear punch and housed in small groups of 2 animals per cage. Animals were acclimatized for at least one week prior to study commencement. During acclimatization, the animals were observed daily in order to reject animals that present poor condition.

3.3 Housing

Th study was performed in animal rooms provided with filtered air at a temperature of 70°F+/-5° F and 50 +/-20% relativ humidity. Animal rooms were set to maintain a minimum of 12 to 15 air

changes per hour. The room was on an automatic timer for a light – dark cycle of 12 hours on and 12 hours off with no twilight.

Hardwood shavings (Aspen) from Northeast Bedding Supply were used. Bedding was packaged in vacuum-packaged bags, irradiated prior to and changed a minimum of once per week.

Cages, tops, bottles, etc. were washed with a commercial detergent and allowed to air dry. Prior to use, these items were wrapped and autoclaved. Cage changes were done in a flow hood. A commercial disinfectant was used to disinfect surfaces and materials introduced into the hood.

Floors were swept daily and mopped a minimum of twice weekly with a commercial detergent. Walls and cage racks were sponged a minimum of once per month with a dilute bleach solution.

A cage card or label with the appropriate information necessary to identify the study, dose, animal number and treatment group will mark all cages. The temperature and relative humidity were recorded during the study, and the records retained.

3.4 Diet

Animals were fed with a standard hamster chow and water *ad libitum*.

3.5 Animal Randomization and Allocations

Hamsters were randomly and prospectively divided into seven (7) treatment groups. Each animal was identified by an ear punch corresponding to an individual number. For more consistent identification, ear punch numbering was used rather than tagging, since tags may become dislodged during the course of the study. A cage card will identify each cage or label marked with the study number, treatment group number and animal numbers.

3.6 Mucositis Induction

Mucositis was induced using an acute radiation protocol. A single dose of radiation (35 Gy/dose) was administered to all animals on Day 0. Radiation was g nerated with a 250 kilovolt potential (15-ma) source at a focal distance of 50 cm, hardened with a 0.35 mm Cu filtration system.

Irradiation targeted the left buccal pouch mucosa at a rate of 121.5 cGy/minute. Prior to irradiation, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg). The left buccal pouch was everted, fixed and isolated using a lead shield.

3.7 Dosing and Drug Application

All animals were dosed three times per day by blinded, unobserved placement. A needleless tuberculin syringe, containing 0.1 ml of the test compound, was inserted into the left cheek pouch and the drug deposited into the pouch.

4. MUCOSITIS EVALUATION

Parameters that were measured in these studies were mucositis score, weight change and survival.

4.1 Survival and Weight Change Data Animal deaths occurred during the study and could often be attributed to the adverse effects of anesthesia, usually at the time of irradiation. Significant numbers of animal deaths, not due to anesthesia, are usually due to toxicity of the experimental compound used in the study. In the acute radiation model used throughout this project, 17 of a total of 280 animals died during the course of all 5 studies. This represented a 6% death rate, all of these deaths were attributable to anesthesia effects. No compound used in these studies appeared to show toxicity. Given the absence of any appreciable changes in animal survival due to experimental compounds, no statistical analysis of survival was done for these studies.
A more sensitive measure of low levels of toxicity is an analysis of the weight change patterns for each animal throughout the study. The percent change in animal weights will be presented for each group. The area under the curve for weight changes will be determined for each animal. A t-test will be used to determine significant differences among groups.

4.2 Mucositis Evaluation For the evaluation of mucositis, the animals were anesthetized with an inhalation anesthetic, and the left pouch everted. Mucositis was scored visually by comparison to a validated photographic scale, as shown in Figure 1. The scale ranges from 0 for normal, to 5 for severe ulceration. In descriptive terms, this scale is defined as follows:

Score:	Description:
0	Pouch completely healthy. No erythema or vasodilation
1	Light to severe erythema and vasodilation. No erosion of mucosa
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray due to pseudomembrane. Cumulative size of ulcers should equal about ¼ of the pouch. Severe erythema and vasodilation.
4	Cumulative seize of ulcers should equal about ½ of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth.

A score of 1-2 is considered to represent a mild stage of the disease, whereas a score of 3-5 is considered to indicate moderate to severe mucositis. Following visual scoring, a photograph was taken of each animal's mucosa using a standardized technique. At the conclusion of the experiment, all films were developed and the photographs randomly numbered. At least two independent trained observers will grade the photographs in blinded fashion using the above described scale (blinded scoring).

4.3 Clinical Significance of the Mucositis Scoring System The clinical mucositis score of 3 in hamsters indicates the presence of an ulcer (see Figure 1). Ulceration is the point in the development of mucositis where the physical integrity of the oral mucosa is breached. At the point of ulceration, the hamster becomes susceptible to infection. Both of these factors are observed with human patients. In the clinic, a patient presenting with oral ulcerations often requires hospitalization with analgesic, narcotic and/or antibiotic therapies. The average cost to the healthcare system is significant. Advanced mucositis in humans (with ulcerative sores) often requires the interruption of therapy for patients receiving radiation and, if sepsis occurs, these patients risk death.

If a therapeutic were to significantly reduce the time that a patient with oral mucositis had ulcers, that therapeutic would be of great value to the clinician. In the hamster model of oral mucositis, the duration of scores of 3 or greater is used as a primary measurement of efficacy in mucositis treatment.

4.4 Analysis of Efficacy of Compounds in Treatment of Mucositis The group mean mucositis scores were compared to the control group in each experiment. This comparison provided an effective graphic representation through which the relative effectiveness of each compound was initially determined.

The significance of differences between groups in this study was determined using the Mann Whitney Rank Sum analysis for the daily mucositis scores. Significance of differences in mucositis scores were determined both on a daily basis and as a summation of the entire experiment.

Because the score of 3 has such great clinical importance (see above), the cumulative number of days that an animal has a score of 3 or greater was determined. The significance of group differences in scores of 3 or greater was determined using chi-square analysis. Chi-square difference analysis was done for cumulative scores for the entire study as well as for daily group scores.

5. RESULTS

5.1 ORA-01

5.1.1 Study Rationale This was the first study in which new classes of compounds were analyzed in the acute radiation model. The first three compounds analyzed were cromolyn, a mast cell inhibitor, indomethacin, an NSAID and minocycline, a tetracycline with numerous biological properties. All the studies presented in this report, consist of 56 hamsters divided into seven groups of eight hamsters per group. In this study, each compound was administered at two doses as follows:

Group 1	placebo, distilled water control
Group 2	cromolyn at 20 mg/ml
Group 3	cromolyn at 2 mg/ml
Group 4	minocycline at 1 mg/ml
Group 5	minocycline at 0.1 mg/ml
Group 6	indomethacin at 1 mg/ml
Group 7	indomethacin at 0.1 mg/ml

The compounds were prepared as follows:

5.1.1.1Cromolyn was provided in 23 individual vials of 100 mg per vial. On each day of the study, a single vial was removed from the rack and 5 ml of sterile distilled water added; this tube was labeled Tube 1. Tube 1 was used for the entire day for dosing Group 2. A 1:10 dilution of the cromolyn solution in Tube 1 was made by taking 0.5 ml of the 20 mg/ml solution and adding 4.5 ml of water. This tube was labeled Tube 2 and used to dose the animals of Group 3 for the entire day.

5.1.1.2 Minocycline was provided in 69 individual vials of 2 mg per vial. Minocyclin is relatively unstable in solution; it was therefore made up immediately prior to each dosing (i.e. 3 times per day). For each dosage, on each day of the study, a single vial was removed from the rack and 5 ml of sterile distilled water added; this tube was labeled Tube 3. Tube 3 was used for a single dosing of animals in Group 4. A 1:10 dilution of the Minocyclin solution in Tube 3 was made by taking 0.5 ml of the 1 mg/ml solution and adding 4.5 ml of water. This tube was labeled Tube 4 and used to dose the animals of Group 5 for a single dose. The pH of the stock minocycline solution was approximately 3.5.

5.1.1.3 Indomethacin was provided in 23 individual vials of 5 mg per vial. On each day of the study, a single vial was removed from the rack and 4.88 ml of sterile distilled water added; this tube was labeled Tube 5. The compound was in the form of a slurry at this point. Eighty microliters of 1N NaOH was added to Tube 5 and the compound solubilized with mixing. Finally, 40 microliters of 1N NaOH were added to tube 5 resulting in a pH of 7.0. Tube 5 was used for the entire day for dosing Group 6. A 1:10 dilution of the Indomethacin solution in Tube 5 was made by taking 0.5 ml of the 1 mg/ml solution and adding 4.5 ml of water. This tube was labeled Tube 6 and used to dose the animals of Group 7 for the entire day.

5.1.2 Study Characteristics (Figure 2). Signs of mucositis began in the placebo group on day 10 and peaked on day 14. The mean peak mucositis score for the placebo group was 3.8 with a SEM of +/-0.15 MU. From day 6 to day 28, the placebo animals had a mucositis score of 3 or greater on 63% of the days analyzed.

Survival was excellent among all groups. A single death occurred on day 0 and was attributable to anesthesia overdose.

5.1.3 Weight Chang_ (Figures 3 & 4). Control animals demonstrated progressive weight gain from day -1 to day 10. From day 10 to day 13, essentially no changes in weight were seen. Beginning on day 14 and continuing until the conclusion of the experiment, control animals gained weight in a linear fashion at the rate of about 2% per day.

Of all groups, hamsters treated with 0.1 mg/ml minocycline demonstrated the most dramatic weight gain. This increase, compared to control animals, appeared as early as day 7. From day 7 to day 28, the rate of weight gain in this group exceeded all others (Figure 3B). The 0.1 mg/ml minocycline group had an overall weight gain of 52% compared to 40% for the control hamsters. The area under the curve analysis (Figure 4) indicates that the weight gain shown by this group is significantly greater than that shown by the control group.

Animals that received 1 mg/ml indomethacin demonstrated significant weight gain when compared to the control group (Figure 4). No significant differences in weight change were seen among the other test groups compared to the control group.

5.1.4 Mucositis Scores (Figures 2, 5 & 6; Tables 2 & 3).

5.1.4.1 Minocycline Treatment with topical minocycline at the two doses tested (1 mg/ml and 0.1 mg/ml) markedly reduced the severity of ulcerative mucositis induced by acute radiation (Figure 2). Neither minocycline group developed mean mucositis scores of 3 at any point during the entire course of the study. Ulcerative mucositis, as indicated by a score of 3 or more, was significantly reduced on 5 of 9 possible days when animals were dosed at 1 mg/ml (Figure 5, Table 2). At a dose of 0.1 mg/ml minocycline a significant reduction of ulceration was observed on 9 of 9 possible days. Rank sum analysis of the distribution of scores indicates strongly significant differences in 5 of the 10 days where mucositis was present in the 1.0 mg/ml group . In the group receiving 0.1 mg/ml minocycline, significant rank sum differences were observed 10 of the possible 10 days of mucositis (Table 3). *These results, if translated to human clinical efficacy, would constitute a cure for the disease.*

Hamsters receiving the lower dose of minocycline (0.1 mg/ml) responded more favorably than did hamsters receiving 1.0 mg/ml minocycline. From day 14 to the conclusion of the experiment, mucositis scores for the 0.1 mg/ml group showed an apparent reverse dose response. Both minocycline groups showed a significant reduction in days with scores of 3 or greater when compared with control animals (Figures 5 & 6).

5.1.4.2 Cromolyn No significant difference in longitudinal mucositis scores were seen when cromolyn-treated animals were compared to controls (Figure 2). With the exception of two days (day 12 and day 24), cromolyn treatment did not effect the percentage of animals with daily scores of 3 or greater (Figure 5). An absence of efficacy after treatment with cromolyn was observed at both doses examined.

5.1.4.3 Indomethacin The topical administration of indomethacin appeared to be marginally beneficial during the resolution or healing phase of the study (days 20-26; Figures 2 & 5). Indomethacin when administered at 0.1 mg/ml led to a significant reduction in the number of days of ulceration (Figure 6).

5.1.5 Conclusions

1. Minocycline administered tid, topically in an aqueous solution markedly reduced the severity and course of ulcerative mucositis induced by a single acute dose of radiation.
2. Animals receiving minocycline gained weight more consistently and rapidly than did control hamsters.
3. The effect of minocycline on acute experimental mucositis was inversely dose related.
4. Topically applied indomethacin was of statistically significant, but clinically marginal benefit relative to the severity and course of mucositis. This observation was inversely dose related.
5. Cromolyn failed to demonstrate efficacy under the conditions tested.

5.2 ORA-02

5.2.1 Study Rationale The second study (ORA-02) was performed simultaneously with ORA-01. In this study three additional NSAIDS were studied for their ability to reduce the severity of ulcerative oral mucositis. The compounds tested were flurbiprofen, etodolac and ketorolac. The compounds were administered at two different concentrations as follows:

Group 1	placebo, distilled water control
Group 2	ketorolac at 1.0 mg/ml
Group 3	ketorolac at 0.1 mg/ml
Group 4	flurbiprofen at 1.0 mg/ml
Group 5	flurbiprofen at 0.1 mg/ml
Group 6	todolac at 1.0 mg/ml
Group 7	etodolac at 0.1 mg/ml

The compounds were prepared as follows: Flurbiprofen, k torolac and etodolac were provided in 23 individual vials of 5 mg per vial. On each day of the study, a single vial was removed from the rack and 2.13 ml of a solution of water (2ml) and 1 N NaOH (130 µl) were be added. The tube was vortexed for 10 seconds and 2.87 ml of water added to the tube (final volume 5.0 ml). After further mixing (10-20 seconds) 80-110 µl of 1 N HCl was added to obtain a pH of 6.8 to 7.6.

A 1:10 dilution was made of each solution by taking 0.5 ml of the 1 mg/ml solution and adding 4.5 ml of sterile distilled water. The tubes were labeled as follows:

Tube 1	1.0 mg/ml ketorolac
Tube 2	0.1 mg/ml ketorolac
Tube 3	1.0 mg/ml flurbiprofen
Tube 4	0.1 mg/ml flurbiprofen
Tube 5	1.0 mg/ml etodolac
Tube 6	0.1 mg/ml etodolac

5.2.2 Study Characteristics (Figure 7). The induction of mucositis in the placebo group began on day 12 and reached a peak on day 16. This pattern of mucositis onset was later than that observed in the first study, although it is unlikely that this is meaningful. For the placebo group, the mean peak mucositis score was 4.1 +/- a SEM of 0.15 MU. From day 6 to day 28, the placebo animals had a mucositis score of 3 or greater on 65.6% of the days analyzed.

Survival was excellent among all groups. A death that occurred on day 0 was attributable to anesthesia overdose. A second anesthesia-related death occurred on day 17 in the etodolac (1 mg/ml) group .

5.2.3 Weight Change (Figures 8 & 9). Overall weight gain in this study was about 20% for all groups. The hamsters receiving water as a placebo lost weight until day 2 then gained weight steadily until day 10 (Figure 8). There was a plateau in weight gain from day 11 to day 16 corresponding to the onset of mucositis. Starting on day 17, consistent weight gain was observed for the remainder of the study.

In the case of all the drug treated groups, weight gain paralleled that observed in the placebo group. In general, the weight gain curves of the drug treated animals appeared to be slower and less robust than that observed in the placebo group (Figure 8). This impression was not supported by analysis

of the area under the curve for all groups in this study (Figur 9). In the AUC analysis, there were no significant differences in the overall weight gain observed in any experimental group when compared with the placebo group.

5.2.4 Mucositis Scores (Figures 7, 10 & 11; Tables 4 & 5).

5.2.4.1 Ketorolac At both doses of ketorolac there appeared to be a reduction of early mucositis on days 8 and 10. This difference was significant by rank sum analysis (Table 5). The subsequent course of ulcerative mucositis appeared unaffected by treatment with ketorolac (Figure 7). While there was a significant reduction of mucositis scores on days 14, 18 and 28, these reductions appeared to be the result of biological variability and not a consistent pattern of healing. Chi square analysis of scores greater than 3 in the ketorolac treated hamsters showed no significant differences on a daily basis (Table 4) or for the sum of study days (Figure 11).

5.2.4.2 Flurbiprofen Hamsters treated with either 1mg/ml or 0.1 mg/ml of flurbiprofen showed no significant improvement in the extent or duration of ulcerative mucositis when compared with the placebo group. As observed in the ketorolac treated animals, there was a brief and significant reduction of mucositis scores on days 8 and 10, but no reduction in the peak levels of mucositis and no improvement in the healing of the lesions (Figure 10, Tables 4 & 5).

5.2.4.3 Etodolac The effect of etodolac treatment on the progression was minimal. As with ketorolac and flurbiprofen, an early and significant reduction of mucositis scores was observed on days 8 and 10 in the group treated with 1 mg/ml etodolac (Figure 7, Table 5). This early improvement was reversed by day 14 and the subsequent course of mucositis was unchanged by treatment with etodolac. There was no significant alteration in the clinically important measure of days of ulceration (Table 4, Figures 10 & 11).

5.2.5 Conclusions

1. Treatment of ulcerative oral mucositis with the NSAIDs ketorolac, flurbiprofen and etodolac consistently resulted in a reduction of weight gain when compared with placebo animals. This weight reduction was not statistically significant when analyzed as the area under the curve.
2. The rate of onset of mucositis on days 8 and 10 was significantly delayed by all three NSAID treatments. In all cases, this reduction was reversed by day 14 and the course of ulcerative mucositis was unchanged by treatment with the NSAIDs.

3. The clinically significant measure of animal days with a score of 3 or greater was unchanged by any NSAID treatment.

5.3 ORA-03

5.3.1 Study Rationale The dramatic results of ORA-01 suggested that minocycline might present a curative therapy for oral mucositis as a single agent. In this study, minocycline was tested a second time to determine if the first result was reproducible. In addition, other tetracyclines were tested in parallel to determine if related tetracyclines had efficacy in treating the disease. The compounds tested were minocycline, tetracycline and doxycycline. The compounds were administered as follows:

Group 1	placebo, distilled water control
Group 2	tetracycline at 1.0 mg/ml
Group 3	tetracycline at 0.1 mg/ml
Group 4	minocycline at 1.0 mg/ml
Group 5	minocycline at 0.1 mg/ml
Group 6	doxycycline at 1.0 mg/ml
Group 7	doxycycline at 0.1 mg/ml

Each compound was pre-weighed in individual vials. Immediately prior to dosing, a fresh vial of compound was solubilized in distilled sterile water and applied within 30 minutes of preparation.

5.3.2 Study Characteristics (Figure 12). The induction of mucositis began on day 12 and reached a peak on day 14. The severity of peak mucositis was approximately 4.1, similar to that observed in ORA-01. The resolution of mucositis was faster in this study than in the previous resulting in a reduction in the number of days with a score or 3 or greater. In ORA-01 and ORA-02 the hamsters in the placebo group spent 65.5% and 65.6% of the study days with ulcerative lesions. In ORA-03 the hamsters had ulcers for only 52.6% of the possible days.

Survival in this study was excellent. One hamster died on day 1 due to anesthesia overdose.

5.3.3 Weight Change (Figures 13 & 14). The trend for weight gain for all three tetracyclines was positive when compared to the placebo group (Figure 13). AUC analysis indicated that only the group receiving minocycline at 0.1 mg/ml had significantly greater w ight gain than did the placebo group. Overall weight gain was in the region of 75% of the starting value, significantly

greater than the 20% weight gain observed in ORA-01 and ORA-02. This is probably due to the fact that the animals were smaller (70g-80g on day -1) than those used in the previous studies. As will be seen, the differences in weight and the smaller therapeutic window of this study was not a factor when considering the efficacy of the compounds.

5.3.4 Mucositis Scores (Figures 12, 15 & 16, Tables 6 &7).

5.3.4.1 Minocycline. Both doses of minocycline (1 mg/ml and 0.1 mg/ml) resulted in significant reductions in the severity of oral mucositis when compared to the placebo group (Figures 12, 14 & 16). The rank sum test indicated a significant difference in the mucositis scores for every day from day 12 to day 28 (Table 7). The daily occurrence of scores of 3 or greater was dramatically reduced in both minocycline-treated groups, the 1.0 mg/ml treatment is clearly more effective than the 0.1 mg/ml dose. The dose response relationship was further supported by the cumulative chi-square analysis of animal days of 3 or greater; the animals treated with 1.0 mg/ml minocycline had a 92% reduction in days with ulcerations while the 0.1 mg/ml minocycline dose resulted in a decrease of 81% (Figure 16). These results were consistent with those observed in ORA-01.

5.3.4.2 Tetracycline Both doses of tetracycline (1 mg/ml and 0.1 mg/ml) resulted in significant reductions in the severity of oral mucositis when compared to the placebo group (Figures 12, 14 & 16). The rank sum test indicates a significant difference in the mucositis scores for every day from day 12 to day 28 (Table 7). The daily occurrence of scores of 3 or greater is dramatically reduced in both tetracycline-treated groups; the 1.0 mg/ml treatment is clearly more effective than the 0.1 mg/ml dose. The dose response relationship is further supported by the cumulative chi square analysis of animal days of 3 or greater; the animals treated with 1.0 mg/ml tetracycline had a 79% reduction in days with ulcerations while the 0.1 mg/ml tetracycline dose resulted in a decrease of 82% (Figure 16).

5.3.4.3 Doxycycline Both doses of doxycycline (1 mg/ml and 0.1 mg/ml) resulted in significant reductions in the severity of oral mucositis when compared to the placebo group (Figures 12, 14 & 16). The rank sum test indicates a significant difference in the mucositis scores for every day from day 12 to day 28 (Table 7). The daily occurrence of scores of 3 or greater is dramatically reduced in both tetracycline-treated groups, the 1.0 mg/ml treatment is clearly more effective than the 0.1 mg/ml dose. The dose response relationship is further supported by the cumulative chi square analysis of animal days of 3 or greater; the animals treated with 1.0 mg/ml doxycycline had a 85%

reduction in days with ulcerations while the 0.1 mg/ml doxycycline dose resulted in a decrease of 75%.

5.3.5 Conclusions

1. Each of the tetracyclines tested markedly effected the extent of mucositis in the treated animals. None, however, effected the course of tissue injury. For all groups, treated and control peak mucositis scores were noted on day 14. This was followed by a healing period which reached baseline for the treated animals, but not for the controls.
2. Administration of each of the tetracyclines tested resulted in a significant reduction in percent of animal days with scores of 3 or more. There did not appear to be a difference among the compounds tested. A slight dose response was seen for minocycline and doxycycline, but not for tetracycline.
3. All of the tetracyclines tested favorably effected the percent animals with scores of 3 or more when analyzed on a daily basis. For each compound, this effect appeared to be dose-dependent. Of the materials and doses tested, minocycline at 1 mg/ml appeared to be most efficacious

5.4 ORA-04

5.4.1 Study Rationale To further validate the effectiveness of minocycline in the treatment oral mucositis and to begin to define a possible mechanism for the action of minocycline, ORA-04 was designed to achieve the following goals:

1. To establish a dose response for minocycline.
2. To confirm the earlier observations as to the efficacy of minocycline.
3. To test the efficacy of minocycline in a second laboratory setting with technicians who had not participated in the previous studies.
4. To determine if the favorable effects of minocycline on X-ray therapy -induced mucositis were independent of its role as an antibiotic. For this part of the experiment a non-tetracycline antibiotic, metronidazole was tested in the same dose range that was shown to be effective in the treatment of mucositis by the tetracyclines.

The compounds were administered as follows:

Group 1	placebo, distilled water control
Group 2	minocyclin at 1.0 mg/ml
Group 3	minocycline at 0.1 mg/ml
Group 4	minocyclin at 0.01 mg/ml

Group 5	minocycline at 0.001 mg/ml
Group 6	metronidazole at 1.0 mg/ml
Group 7	metronidazole at 0.1 mg/ml

Each compound was pre-weighed in individual vials. Immediately prior to dosing, a fresh vial of compound was solubilized in distilled sterile water and applied within 30 minutes of preparation.

5.4.2 Study Characteristics (Figure 17). The induction of mucositis began on day 10 and reached a peak on day 16. The severity of peak mucositis was approximately 2.9. The resolution of mucositis was faster in this study than in the previous resulting in a reduction in the number of days with a score or 3 or greater. In ORA-01, ORA-02 and ORA-03 the hamsters in the placebo group spent 65.5%, 65.6% and 52.6% of the study days with ulcerative lesions respectively. In ORA-04 the hamsters had ulcers for only 30.7% of the possible days. This rate of ulcerative mucositis is somewhat lower than is normally observed, but well within an acceptable range for the model.

There were 6 deaths in this study. All were apparently due to anesthesia overdose. Five of the deaths occurred during the irradiation on day 0.

5.4.3 Weight Changes (Figures 18 & 19). The trend for weight gain for all four minocycline concentrations was positive when compared to the placebo group (Figure 18A). AUC analysis indicated that only the group receiving minocycline at 0.1 mg/ml had significantly greater weight gain than did the placebo group. Overall weight gain was in the region of 40-55% of the starting value. Both metronidazole groups exhibited increased weight gain when compared to the placebo group (Figure 18B), but no group differences achieved significance in the AUC analysis (Figure 19). As in ORA-03, the differences in weight and the smaller therapeutic window of this study was not a factor when considering the efficacy of the compounds.

5.4.4 Mucositis Scores (Figures 17, 20 & 21; Tables 8 & 9).

5.4.4.1 Minocycline. In this dose response experiment, there was a clear lower limit for the efficacy of minocycline in the treatment of ulcerative mucositis. Both of the higher doses of minocycline (1 mg/ml and 0.1 mg/ml) resulted in significant reductions in the severity of oral mucositis when compared to the placebo group (Figure 17A). The rank sum test indicated a significant reduction in the mucositis scores for 7 of 10 possible day in the group treated with 1 mg/ml minocycline and in 8 of 10 possible days for the group treated with 0.1 mg/ml minocycline (Table 9). The daily

occurrence of scores of 3 or greater was dramatically reduced in both minocycline-treated groups, the 1.0 mg/ml treatment is slightly more effective than the 0.1 mg/ml dose (Table 8, Figure 20 A & B). The cumulative chi-square analysis of animal days of 3 or greater showed that the animals treated with 1.0 mg/ml minocycline had a 98% reduction in days with ulcerations while the 0.1 mg/ml minocycline dose resulted in a decrease of 92%. These results were consistent with those observed in ORA-01 and ORA-03.

At the two lower doses of minocycline (0.01 mg/ml and 0.001 mg/ml) the efficacy in treatment of mucositis was not observed (figure 17B). A single day of significant reduction of mucositis scores was observed on day 10, prior to the onset of ulceration. After day 10 there was no day on which the groups receiving the lower concentrations of minocycline showed either significant reduction of mucositis score (Table 9) or reduction in the extent of ulceration (Table 8). The lower doses of minocycline appeared to worsen the resolution of mucositis (Figure 17B) and, in the case of the group treated with 0.001 mg/ml minocycline, the increase of severity of mucositis was significant in the analysis of cumulative days spent with ulcerations (Figure 21).

5.4.4.2 Metronidazole. The non-tetracycline antibiotic metronidazole was not effective in reducing the severity of oral mucositis. Analysis of the mean group scores (Figure 17C) indicates a worsening of mucositis at both the 1 mg/ml and 0.1 mg/ml doses. By rank sum analysis of scores, the 1 mg/ml dose resulted in a significant worsening of mucositis from day 18 to day 28 (Table 9). This observation is confirmed by the analysis of clinical scores of 3 or more both on a daily basis (Figure 20E) and when summarized over the entire study (Figure 21).

5.4.5 Conclusions

1. Minocycline at doses of 0.01 mg/ml and 0.001 mg/ml are ineffective in altering the course of mucositis.
2. Minocycline at doses of 1 mg/ml and 0.1 mg/ml are equivalent and both significantly lower mucositis scores compared to the placebo group.
3. Neither metronidazole doses tested showed any efficacy in treating mucositis. At 1 mg/ml, metronidazole significantly worsened the course of the disease. Metronidazole at 1 mg/ml improved weight gain when compared to the placebo group.
4. Minocycline at 0.1 mg/ml was the only dose that favorably modified weight change secondarily to X-ray therapy. The other doses of minocycline (above and below 0.1 mg/ml) had no effect on weight change.

5. Based on observations 2 and 4, it is possible that 0.1 mg/ml minocycline is near the optimum dose

5.5 ORA-05

5.5.1 Study Rationale Study ORA-05 was designed to confirm the efficacy of minocycline as observed in three previous studies. In addition, this study was designed to test the hypothesis that a combination of a tetracycline and an NSAID might provide additional efficacy in the treatment of oral mucositis. Flurbiprofen was chosen as the NSAID. A possible explanation of the failure of NSAIDS to show efficacy in the treatment of mucositis in studies ORA-01 and ORA-02 was the inappropriate formulation of the compounds. In this study, the flurbiprofen was not be solubilized by altering the pH, but was delivered as a suspension in a cyclodextrin vehicle. To accomplish this, a vehicle, methylbeta-cyclodextrin, was used at concentration of 150 mg/ml in all solutions.

The compounds were administered as follows:

Group 1	no vehicle control, distilled water control
Group 2	vehicle control; 150 mg/ml cyclodextrin in water
Group 3	minocycline at 0.1 mg/ml in cyclodextrin (made fresh before application)
Group 4	flurbiprofen at 20 mg/ml in cyclodextrin
Group 5	flurbiprofen at 2 mg/ml in cyclodextrin
Group 6	flurbiprofen at 20 mg/ml + minocycline at 0.1 mg/ml in cyclodextrin
Group 7	flurbiprofen at 2 mg/ml + minocycline at 0.1 mg/ml in cyclodextrin

5.5.2 Study Characteristics (Figure 22). In this study the onset of mucositis was on day 12 with a peak mucositis score of 3.4 occurring in the no vehicle control on day 16. Comparison of the no vehicle control with the vehicle control indicates a significant difference in score on day 16 (Table 11). The control groups were statistically the same for all other days of the study. Thus, the vehicle itself had little effect on the course of oral mucositis. In all subsequent analysis, the treated groups are compared with the vehicle control group.

5.5.3 Weight Changes (Figures 23 & 24). The trend for weight gain for all five test compounds was positive when compared to either control group (Figure 23). AUC analysis indicated that the group receiving flurbiprofen at 20 mg/ml gained significantly less weight than did the control groups (Figure 24). In the same analysis, the group receiving a combination of 2 mg/ml flurbiprofen and

0.1 mg/ml minocycline gained significantly more weight than did the control groups. Overall weight gain was in the region of 50-75% of the starting value.

5.5.4 Mucositis Scores (Figures 22, 25 & 26; Tables 10 & 11).

5.5.4.1 Minocycline. The predicted efficacy of minocycline at 0.1 mg/ml was clearly observed in this study (Figure 22B). Significant reduction of mucositis score was observed on 7 of 9 possible days (Table 11) and reduction of ulceration was observed on 4 of 8 possible days Figure 25B, Table 10). Over the course of the entire study, treatment with 0.1 mg/ml minocycline resulted in an 80% reduction in days with a score of 3 or more (Figure 26). These results are consistent with those observed in three previous studies (ORA-01, ORA-03 & ORA-04)

5.5.4.2 Flurbiprofen. As a single agent, flurbiprofen, when applied at 20 mg/ml or 2 mg/ml, showed no efficacy in the treatment of oral mucositis (Figure 22C). A significant increase in mucositis scores was observed on 2 of 9 possible days for each dose group (Table 11). Both doses of flurbiprofen significantly increased the number of days with a score of 3 or more (Figure 25C & D, Figure 26). Thus the proposed benefit in treatment of mucositis with flurbiprofen was not observed in this study.

5.5.4.3 Combinations of flurbiprofen and minocycline. This study was designed to test the hypothesis that a combination of minocycline and the NSAID flurbiprofen would provide an additional efficacy over the use of either compound alone. Based on the mean mucositis scores of the group treated with 20 mg/ml flurbiprofen and 0.1 mg/ml minocycline, and the group treated with 2 mg/ml flurbiprofen and 0.1 mg/ml minocycline (Figure 22D) the combinations both failed to show and efficacy in this model.

The group treated with 2 mg/ml flurbiprofen and 0.1 mg/ml minocycline showed no significant difference from the vehicle control group on any day in any analysis (Figures 25F & 26, Tables 10 & 11). Thus, while minocycline alone was curative in this study, the addition of flurbiprofen masked the beneficial effects of minocycline. The group treated with 20 mg/ml flurbiprofen with 0.1 mg/ml minocycline caused significant worsening of mucositis both in terms of increasing the score (Table 11) and increasing the days with ulcerations (Figures 25E & 26, Table 10). In both combination doses, there is no indication of synergy; in fact th re is no evidence of additivity.

5.5.5 Conclusions

1.This study confirms that minocycline at a dose of 0.1 mg/ml effectively and dramatically attenuates X-ray therapy-induced mucositis. As noted previously, administration of minocycline does not alter the course of the condition. Peak mucositis is noted on day 14 for both the treated and control animals. The severity of peak mucositis is favorably modulated by minocycline and resolution of the condition is more rapid in the minocycline-treated hamsters.

2. Flurbiprofen, at both doses tested, failed to modulate the course or severity of mucositis.

3. Addition of flurbiprofen to minocycline eliminated the efficacy of minocycline. Furthermore, minocycline plus high dose (20 mg/ml) flurbiprofen resulted in a course of mucositis that was more severe than was seen in control hamsters.

5.6 METANALYSIS OF MINOCYCLINE EFFICACY

5.6.1 Rationale In this report, we have described 5 separate studies where the acute radiation model for oral mucositis in hamsters. Each study had an identical protocol regarding the procedural events of dosing, radiation, and documentation. In addition, after the demonstration of efficacy of minocycline in ORA-01, the dose and schedule used for minocycline was repeated in two additional studies (ORA-03 and ORA-04). In this section we present a metanalysis of the three groups common to each of these studies: placebo, minocycline at 1 mg/ml and minocycline at 0.1 mg/ml. The results of ORA-05 were not included in this analysis as the inclusion of a vehicle in that study altered the protocol.

In each of the previous studies there was a slightly different weight change pattern depending on the study. The results of each study indicate that there is little correlation between weight change and the course of mucositis in this model. Because the hamster is irradiated in only one cheek pouch, the ability to eat is not significantly impaired. Minocycline treatment, at the doses studied here, had no apparent toxicity. For these reasons, we will not consider weight change as part of this metanalysis.

In this analysis, the blinded mucositis scores from ORA-01, ORA-03 and ORA-04 were summed for each day of the study. The mean mucositis scores are shown in Figure 27. The daily mucositis scores of 3 or more, with attendant chi-square analysis, are shown in Figure 28, and the summary chi-square analysis of total days with a score of 3 or more is shown in Figure 29.

5.6.2 Mucositis Scores (Figures 27-29)

A summary of the daily mucositis scores resulted in a composite mucositis profile of the placebo group where mucositis begins on day 10 and peaks on day 14 with a peak score of 3.5 +/- a SM of 0.15 MU. Mucositis declines gradually until day 28 where partial resolution is observed at a score of 1.6 (Figure 27). In both groups treated with minocycline there is a dramatic reduction in the onset and severity of mucositis with a peak score in both groups of 2.3. In this analysis, the 1 mg/ml group appears to resolve more rapidly than the group receiving 0.1 mg/ml minocycline. Rank sum analysis of the blinded mucositis scores indicates that at both concentrations of minocycline, all possible day of mucositis (10 of 10) show a highly significant reduction in mucositis scores.

In the clinical analysis of daily scores of 3 or more both dosing groups showed significant reductions in ulcerations for every possible day of the study (9 of 9, Table 12, Figure 28). The summation of days with scores of 3 or more shows a 79% reduction of ulcerative days in the animals treated with 1 mg/ml minocycline and an 88% reduction in ulcerations in animals receiving 0.1 mg/ml minocycline (Figure 29). All the scores for all days of mucositis in this analysis show a high level of statistical significance.

5.6.3 Conclusions

1. A metanalysis of three studies of topical application of minocycline at doses of 1 mg/ml and 0.1 mg/ml showed complete efficacy at both doses.
2. There was little or no dose response between the two doses of minocycline used, they were similar in terms of efficacy. The 0.1 mg/ml dose may prove to be optimal for the topical treatment of the disease.

7. PROJECT CONCLUSIONS

1. Minocycline, used topically three times a day, at a dose of 1 mg/ml and 0.1 mg/ml, in a volume of 0.1 ml, provides a significantly efficacious treatment for radiation-induced oral mucositis in hamsters.

2. The efficacy of minocycline is produced by other tetracyclines, particularly tetracycline and doxycycline. Efficacy is observed at similar doses for all tetracyclines tested to date.

3. Doses of minocycline below 0.1 mg/ml, particularly 0.01 and 0.001 mg/ml did not show efficacy in treating mucositis.

4. NSAIDs, including flurbiprofen, indomethacin, etodolac and ketorolac, when applied as topical solutions at neutral pH did not show any benefit in the treatment of oral mucositis.

5. The mast cell inhibitor cromolyn, when applied topically, did not show efficacy in the treatment of oral mucositis.

6. The antibiotic metranidazole, when applied topically at 1 mg/ml and 0.1 mg/ml did not demonstrate efficacy in treating oral mucositis.

7. A combination of minocycline (0.1 mg/ml) and flurbiprofen at either 20 mg/ml or 2 mg/ml failed to show efficacy in treating oral mucositis. The combinations were not additive with respect to the activities of either agent applied alone. This result suggests that other 'cocktail' treatments for mucositis that included a tetracycline may have failed to observe appropriate efficacy form the compound.

Protocol for Orapharma Studies ORA-01 through ORA-05

Task	Day																												
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Randomize	X																													
Number Animals	X																													
W igh	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Irradiate		X																												
Apply Compound	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							
Clinical Mucositis Score								X		X		X		X		X		X		X		X		X		X		X		X
Photograph Buccal Pouch								X		X		X		X		X		X		X		X		X		X		X		X
R cord Behavior & Survival	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 1. This is a flow diagram indicating the timing of the tasks associated with each study described in this report.

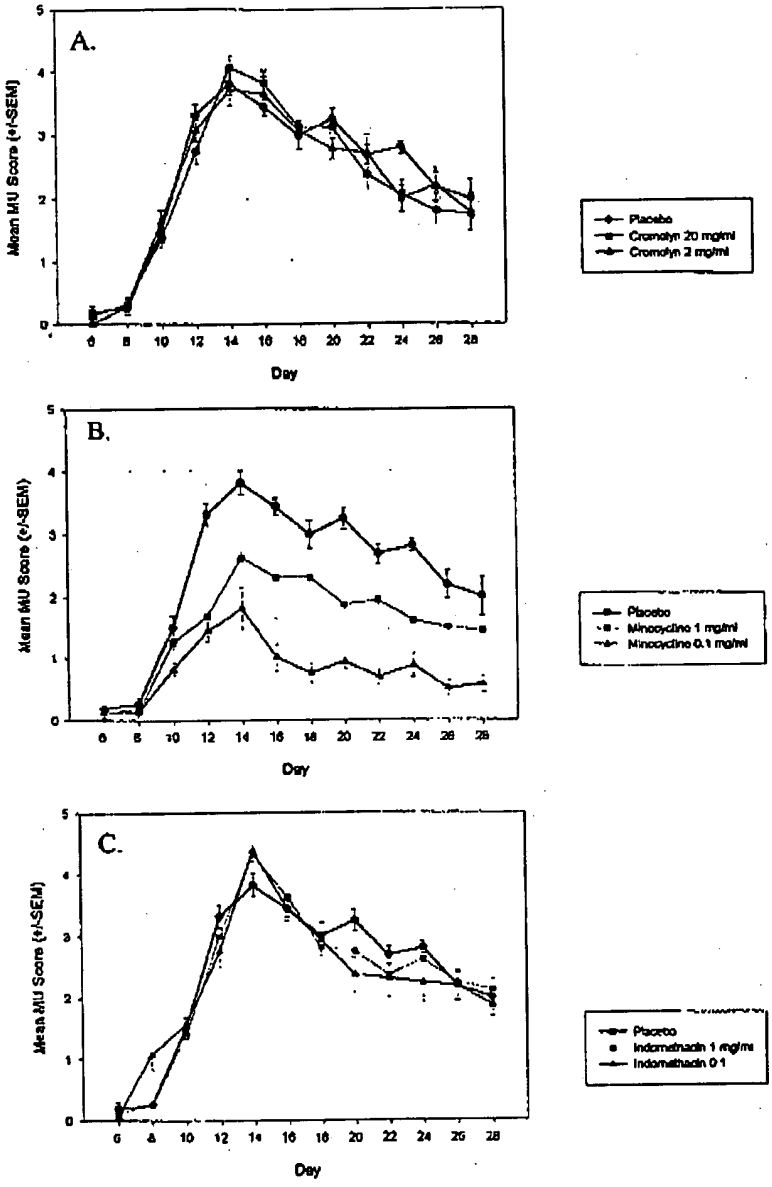


Figure 2. ORA-01. Mean group mucositis scores were obtained for the animal groups receiving topical treatment in water (placebo). Error bars represent the standard error of the mean (SEM). A. Comparison of the placebo group with groups receiving cromolyn at either 20 mg/ml or 2 mg/ml. B. Comparison of the placebo group with animals receiving minocycline at either 1 mg/ml or 0.1 mg/ml. C. Comparison of the placebo group with animals receiving indomethacin at either 1 mg/ml or 0.1 mg/ml.

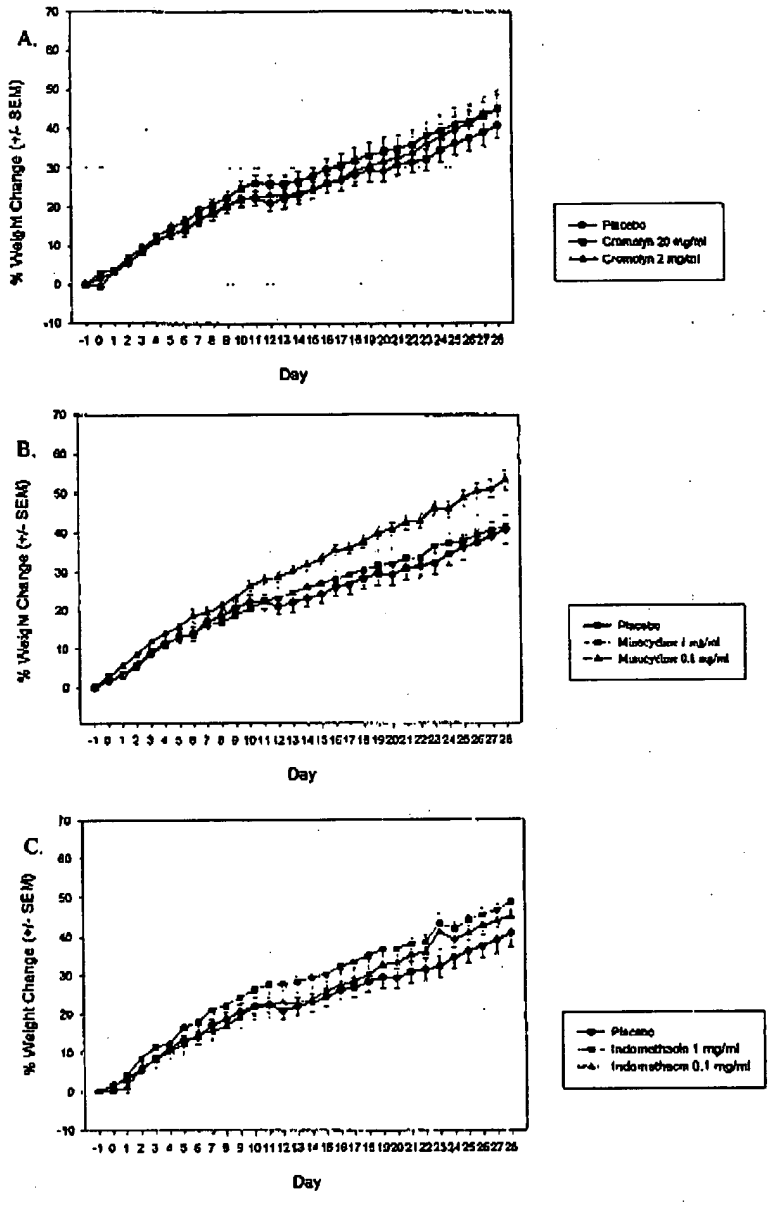


Figure 3. ORA-01. Percent weight change. Animals were weighed daily and group means and standard errors of the mean (SEM) calculated for each day. A. Compares weight gain trends for the groups receiving cromolyn at 20 mg/ml and 2 mg/ml with the placebo group. B. Compares the placebo group with groups receiving minocycline at 1 mg/ml and 0.1 mg/ml. C. Compares the placebo group with groups receiving indomethacin at 1 mg/ml and 0.1 mg/ml.

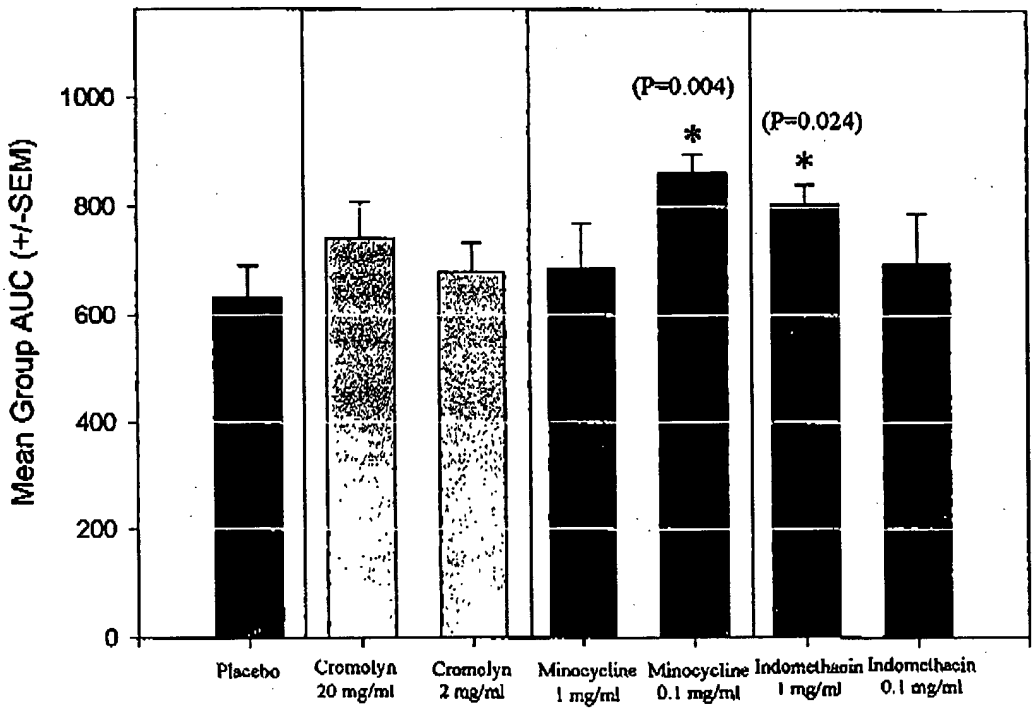


Figure 4. ORA-01. The area under the curve (AUC) was calculated for the percent weight change exhibited by each animal in the study. This calculation was made using the trapezoidal rule transformation. Group means were calculated and are shown with error bars representing SEM for each group. An unpaired t-test was done to compare these groups. Both the minocycline at 0.1 mg/ml and the indomethacin at 1 mg/ml groups gained significantly more weight gain than the control groups.

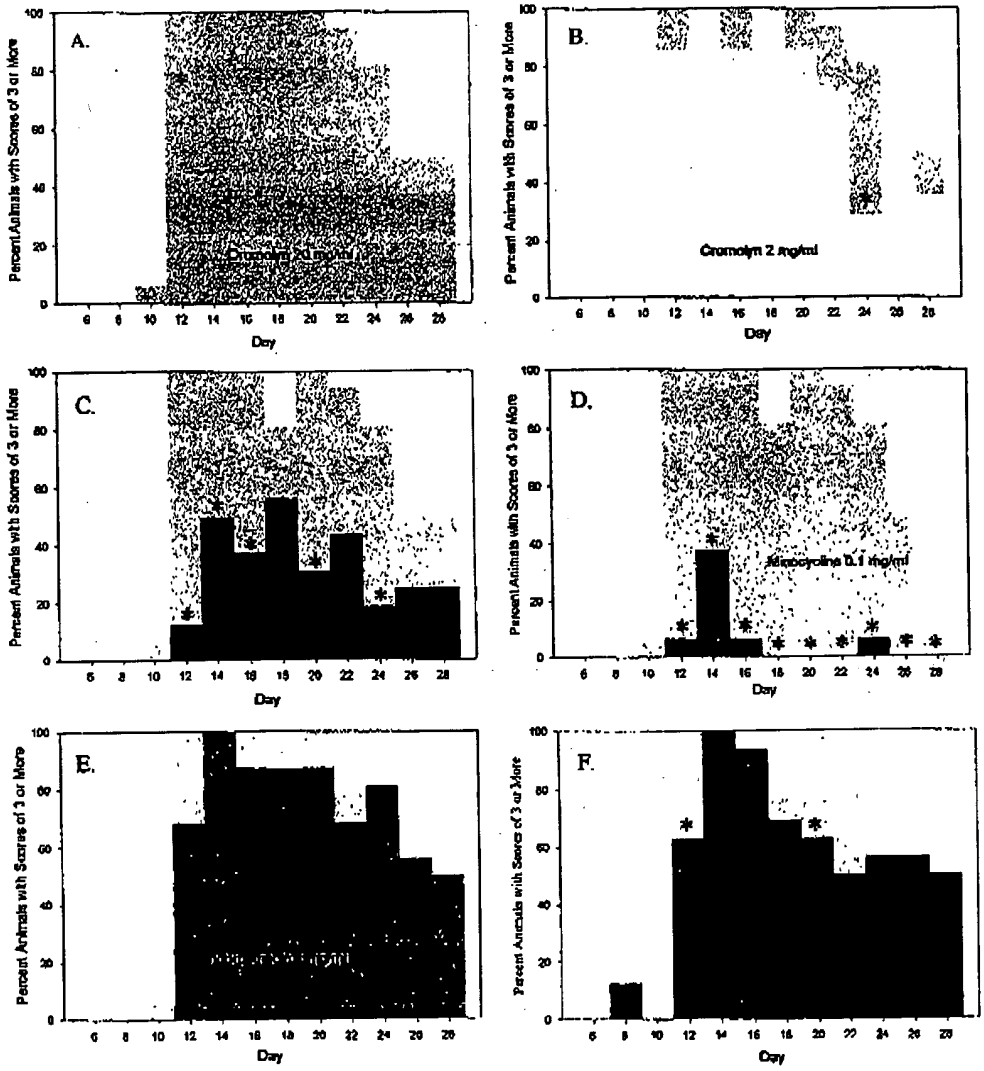


Figure 5. ORA-01. Daily mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the percentage of animals with a score of 3 or more was determined daily for each group. The colored plot represents the drug-treated group while the gray plot represents the placebo group. Statistical significance of the daily observed differences was calculated using chi-square analysis, significant differences between placebo and treated groups are indicated by asterisks. Treatment with cromolyn at 20 mg/ml (A) and 2 mg/ml (B) had little effect on the course of mucositis. Minocycline treatment resulted in a large, highly significant reduction in ulcerative mucositis at both 1 mg/ml (C) and 0.1 mg/ml (D). Indomethacin treatment had no apparent effect on the course of mucositis at 1 mg/ml (E). There appears to be a slight reduction of mucositis at 0.1 mg/ml indomethacin (F).

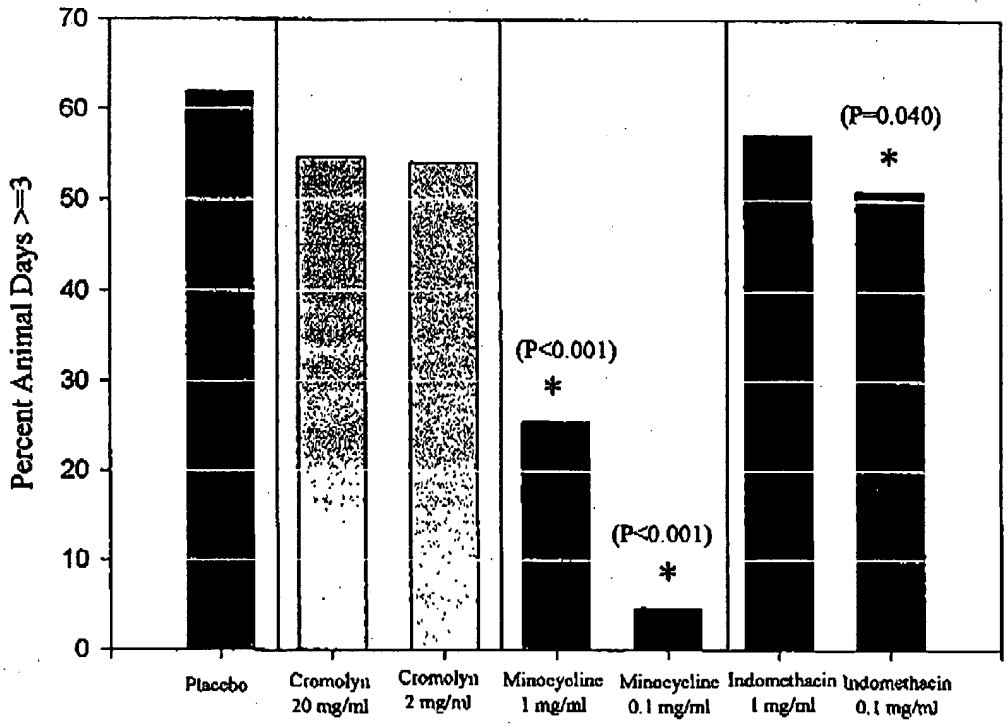


Figure 6. ORA-01. Animal days with mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the total number of days in which an animal exhibited an elevated score were summed and expressed as a percentage of the total number of days. Statistical significance of observed differences was calculated using chi-square analysis. Asterisks indicate significant differences between individual treatment groups and placebo animals. Both minocycline-treated groups had a significantly lower percentage of days with ulcers.

ORA-01 Chi Square Analysis of Daily Mucositis Scores >=3 (P Values)
Significant Scores in Red

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Placebo v 20 mg/ml Chromalyn	1.000	1.000	1.000	0.043	1.000	1.000	0.226	1.000	0.703	0.068	0.722	0.722
Placebo v 2 mg/ml Chromalyn	1.000	1.000	1.000	0.209	1.000	0.209	0.602	0.209	1.000	0.011	0.98	0.676
Placebo v 1 mg/ml Minocycline	1.000	1.000	1.000	<0.001	0.012	<0.001	0.253	<0.001	0.150	0.001	0.273	0.273
Placebo v 0.1 mg/ml Minocycline	1.000	1.000	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.002
Placebo v 1 mg/ml Indomethacin	1.000	1.000	1.000	0.043	1.000	0.484	1.000	0.484	1.000	1.000	1.000	1.000
Placebo v 0.1 mg/ml Indomethacin	1.000	0.484	1.000	0.018	1.000	1.000	0.685	0.018	0.273	0.253	1.000	1.000

Table 2. Chi-square analysis of the distribution of daily scores. The percent distrubution of scores of 3 or more compared to scores less than 3 is shown in Figure 5. The significance of differences observed in that analysis is determined by Chi-square analysis. Values shown here are P values for each calculation. Significance is defined as a P value less than or equal to 0.050.

ORA-01 Mann-Whitney Rank Sum Test of Mucositis Scores (P Values)
Significant Scores in Red

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Placebo v 20 mg/ml Chromalyn	0.775	0.776	0.533	0.112	0.398	0.265	0.663	0.854	0.437	0.033	0.375	0.558
Placebo v 2 mg/ml Chromalyn	0.388	0.884	0.662	0.601	0.662	0.505	0.818	0.248	0.692	0.007	0.868	0.588
Placebo v 1 mg/ml Minocycline	0.985	0.556	0.298	<0.001	<0.001	<0.001	0.072	<0.001	0.054	<0.001	0.101	0.199
Placebo v 0.1 mg/ml Minocycline	0.775	0.556	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
Placebo v 1 mg/ml Indomethacin	0.775	0.985	0.61	0.316	0.097	0.299	0.91	0.261	0.609	0.924	0.85	0.865
Placebo v 0.1 mg/ml Indomethacin	0.555	0.016	0.806	0.192	0.057	0.85	0.925	0.056	0.417	0.325	0.97	0.867

Table 3. The significance of group differences differences observed in daily mucositis scores was determined using the Mann-Whitney rank sum test. This nonparametric statistic is appropriate for the visual mucositis scoring scale. The P values for each calculation are shown.

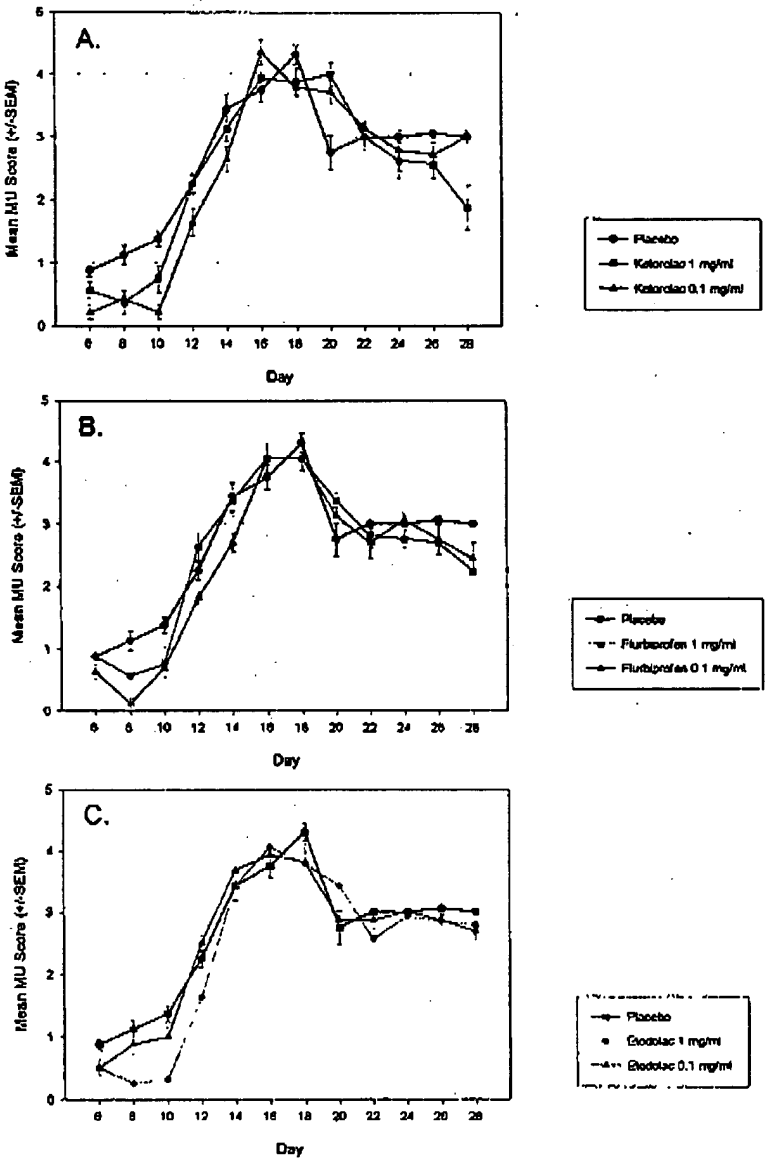


Figure 7. ORA-02. Mean group mucositis scores were obtained for the animal groups receiving topical treatment in water (placebo). Error bars represent the standard error of the mean (SEM). A. Comparison of the placebo group with groups receiving ketorolac at either 1 mg/ml or 0.1 mg/ml. B. Comparison of the placebo group with animals receiving flurbiprofen at either 1 mg/ml or 0.1 mg/ml. C. Comparison of the placebo group with animals receiving etodolac at either 1 mg/ml or 0.1 mg/ml.

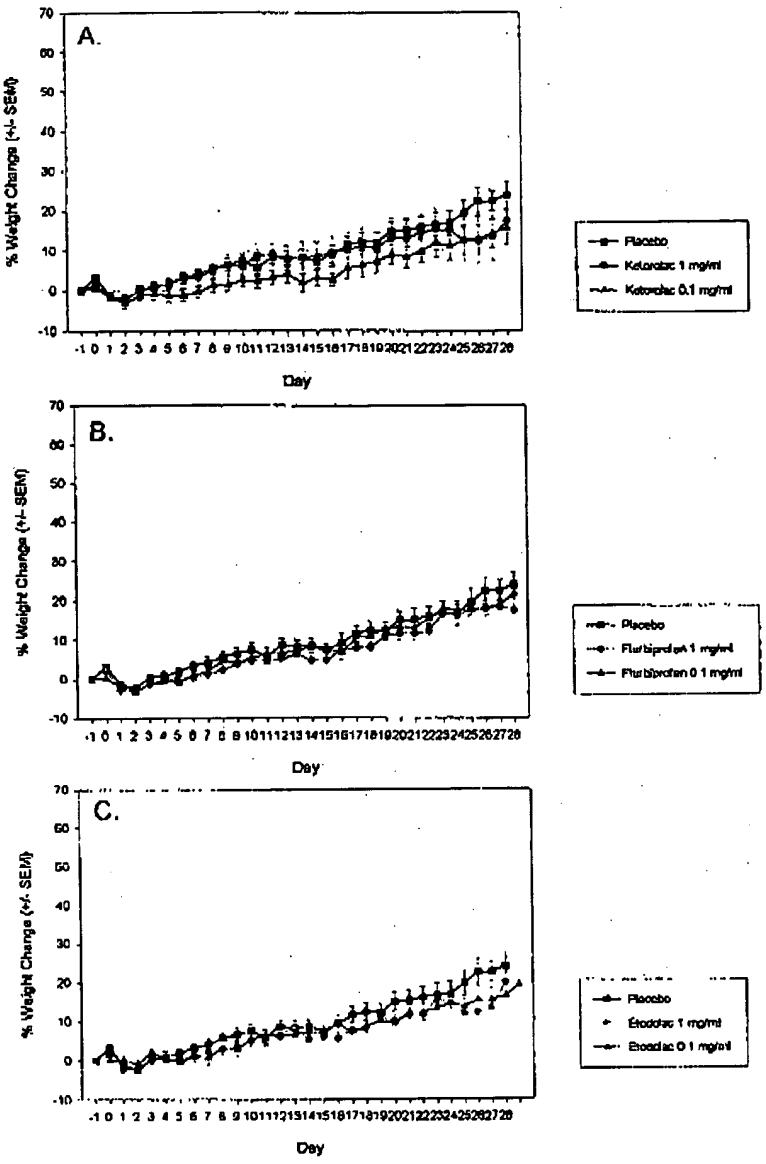


Figure 8. ORA-02. Percent weight change. Animals were weighed daily and group means and standard errors of the mean (SEM) calculated for each day. A. Compares weight gain trends for the groups receiving ketorolac at 1 mg/ml and 0.1 mg/ml with the placebo group. B. Compares the placebo group with groups receiving flurbiprofen at 1 mg/ml and 0.1 mg/ml. C. Compares the placebo group with groups receiving etodolac at 1 mg/ml and 0.1 mg/ml.

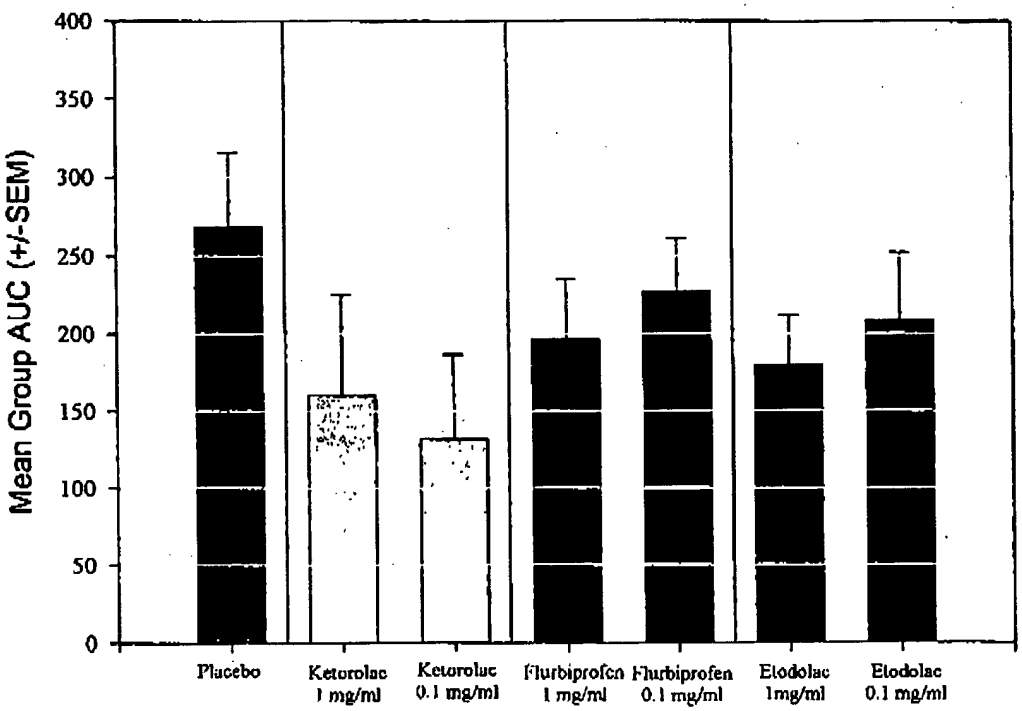


Figure 9. ORA-02. The area under the curve (AUC) was calculated for the percent weight change exhibited by each animal in the study. This calculation was made using the trapezoidal rule transformation. Group means were calculated and are shown with error bars representing SEM for each group. An unpaired t-test was done to compare these groups. There were no significant differences between any treated group and the placebo group in this study.

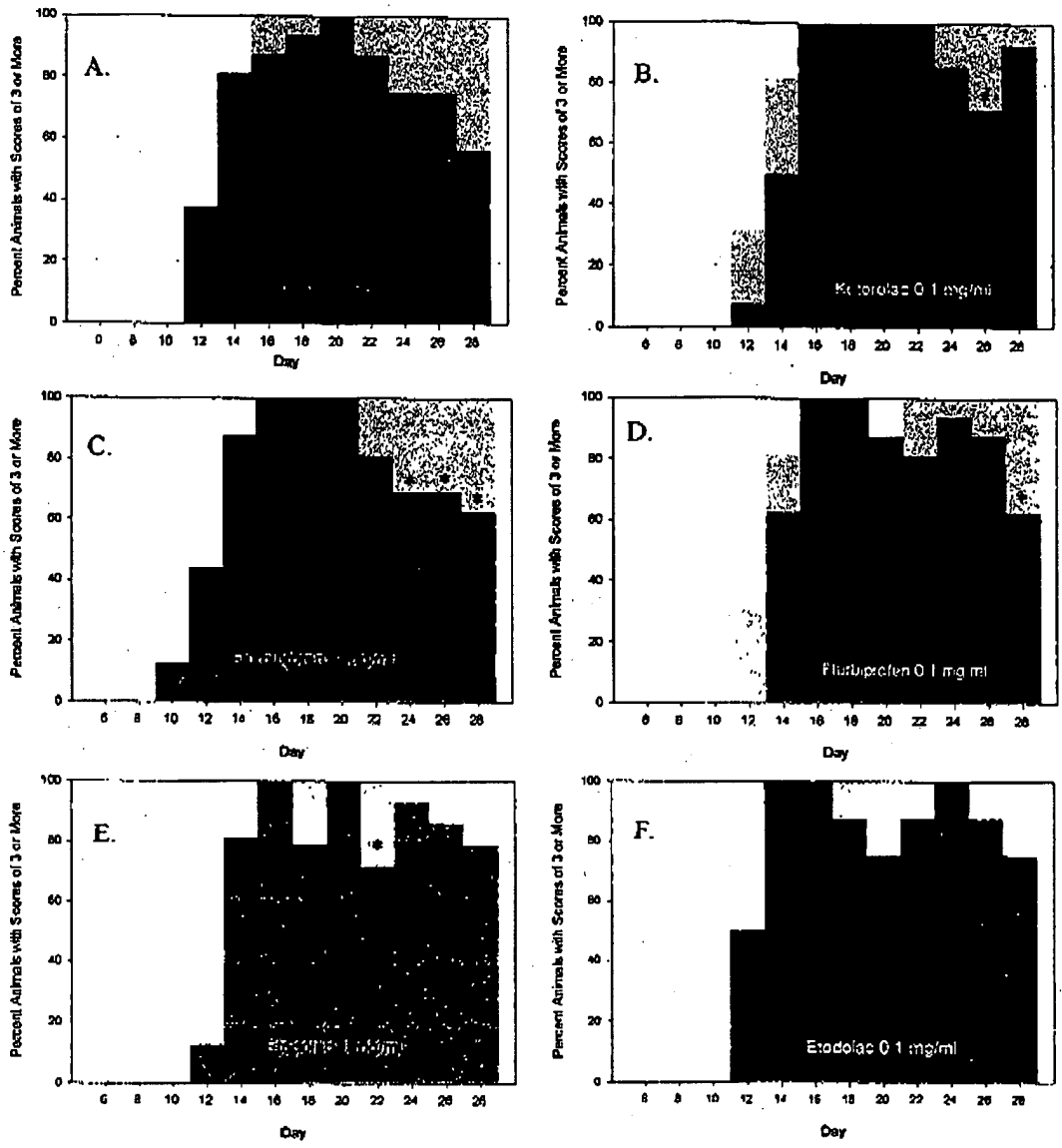


Figure 10. ORA-02. Daily mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the percentage of animals with a score of 3 or more was determined daily for each group. The colored plot represents the drug-treated group while the gray plot represents the placebo group. Statistical significance of the daily observed differences was calculated using chi-square analysis, significant differences between placebo and treated groups are indicated by asterisks. Treatment with ketorolac at 1 mg/ml (A) and 0.1 mg/ml (B) had little effect on the course of mucositis. Flurbiprofen treatment resulted in a small, but significant reduction in ulcerative mucositis at 1 mg/ml (C). The flurbiprofen treatment at 0.1 mg/ml did not significantly alter mucositis (D). Etodolac treatment had no major effect on the course of mucositis at 1 mg/ml (E) or at 0.1 mg/ml (F).

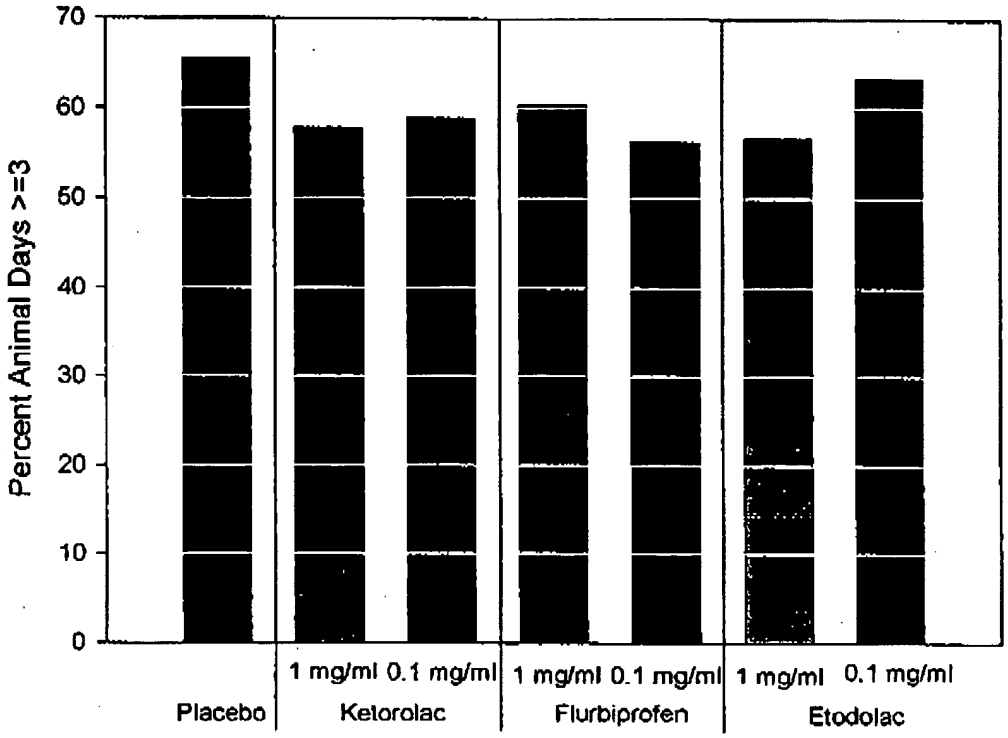


Figure 11. ORA-02. Animal days with mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), The total number of days in which an animal exhibited an elevated score were summed and expressed as a percentage of the total number of days. Statistical significance of observed differences was calculated using chi-square analysis. There were no significant differences between any two groups in this study.

ORA-02 Chi Square Analysis of Daily Mucositis Scores >=3 (P Values)
Significant Scores in Red

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Placebo v 1 mg/ml Ketorolac	1.000	1.000	1.000	NS	1.000	0.484	1.000	0.101	0.484	0.101	0.101	0.007
Placebo v 0.1 mg/ml Ketorolac	1.000	1.000	1.000	0.175	0.122	1.000	1.000	0.103	1.000	0.209	0.037	0.467
Placebo v 1 mg/ml Flurbiprofen	1.000	1.000	0.484	0.715	1.000	1.000	1.000	0.101	0.226	0.043	0.043	0.007
Placebo v 0.1 mg/ml Flurbiprofen	1.000	1.000	1.000	[illegible]	0.433	1.000	1.000	0.654	0.226	1.000	0.484	0.018
Placebo v 1 mg/ml Etodolac	1.000	1.000	1.000	0.394	1.000	1.000	0.090	0.103	0.037	0.467	0.209	0.090
Placebo v 0.1 mg/ml Etodolac	1.000	1.000	1.000	0.472	0.226	1.000	0.484	1.000	0.484	1.000	0.484	0.101

Table 4. Chi-square analysis of the distribution of daily scores. The percent distrubution of scores of 3 or more compared to scores less than 3 is shown in Figure 10. The significance of differences observed in that analysis is determined by Chi-square analysis. Values shown here are P values for each calculation. Significance is defined as a P value less than or equal to 0.050.

ORA-02 Mann Whitney Rank Sum Test of Daily Mucositis Scores (P Values)
Significant Efficacy in Red, Significant Worsening in Green

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Placebo v 1 mg/ml Ketorolac	0.134	0.006	0.036	0.955	0.336	0.428	0.220	0.001	0.556	0.556	0.154	0.035
Placebo v 0.1 mg/ml Ketorolac	0.062	0.010	<0.001	0.073	0.032	0.055	0.039	0.013	0.753	0.753	0.232	0.983
Placebo v 1 mg/ml Flurbiprofen	0.985	0.036	0.025	0.344	0.925	0.290	0.406	0.067	0.231	0.231	0.160	0.035
Placebo v 0.1 mg/ml Flurbiprofen	0.232	<0.001	0.008	0.069	0.029	0.365	0.461	0.067	0.775	0.775	0.581	0.134
Placebo v 1 mg/ml Etodolac	0.072	<0.001	<0.001	0.056	0.970	0.227	0.382	0.052	0.324	0.752	0.366	0.323
Placebo v 0.1 mg/ml Etodolac	0.072	0.335	0.145	0.298	0.588	0.521	0.290	0.865	0.775	0.985	0.391	0.230

Table 5. The significance of group differences differences observed in daily mucositis scores was determined using the Mann-Whitney rank sum test. This nonparametric statistic is appropriate for the visual mucositis scoring scale. The P values for each calculation are shown.

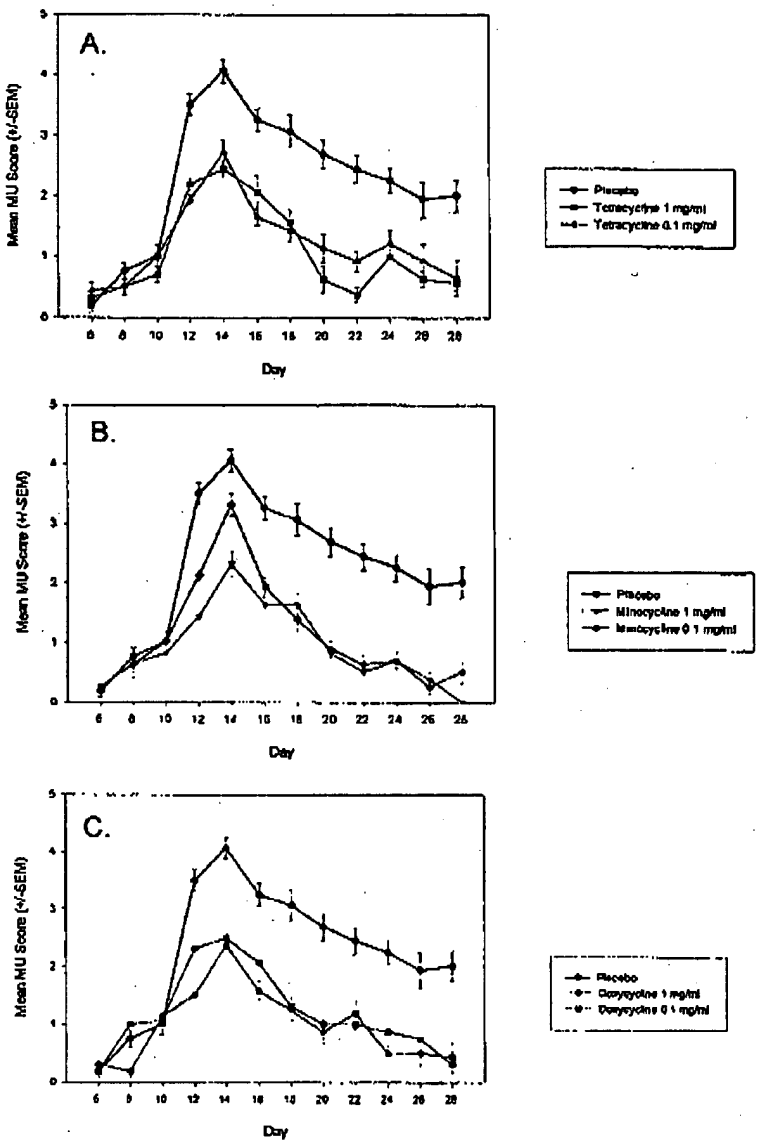


Figure 12. ORA-03. Mean group mucositis scores were obtained for the animal groups receiving topical treatment in water (placebo). Error bars represent the standard error of the mean (SEM). A. Comparison of the placebo group with groups receiving tetracycline at either 1 mg/ml or 0.1 mg/ml. B. Comparison of the placebo group with animals receiving minocycline at either 1 mg/ml or 0.1 mg/ml. C. Comparison of the placebo group with animals receiving doxycycline at either 1 mg/ml or 0.1 mg/ml. In every treatment group a great reduction in mucositis severity and duration was observed.

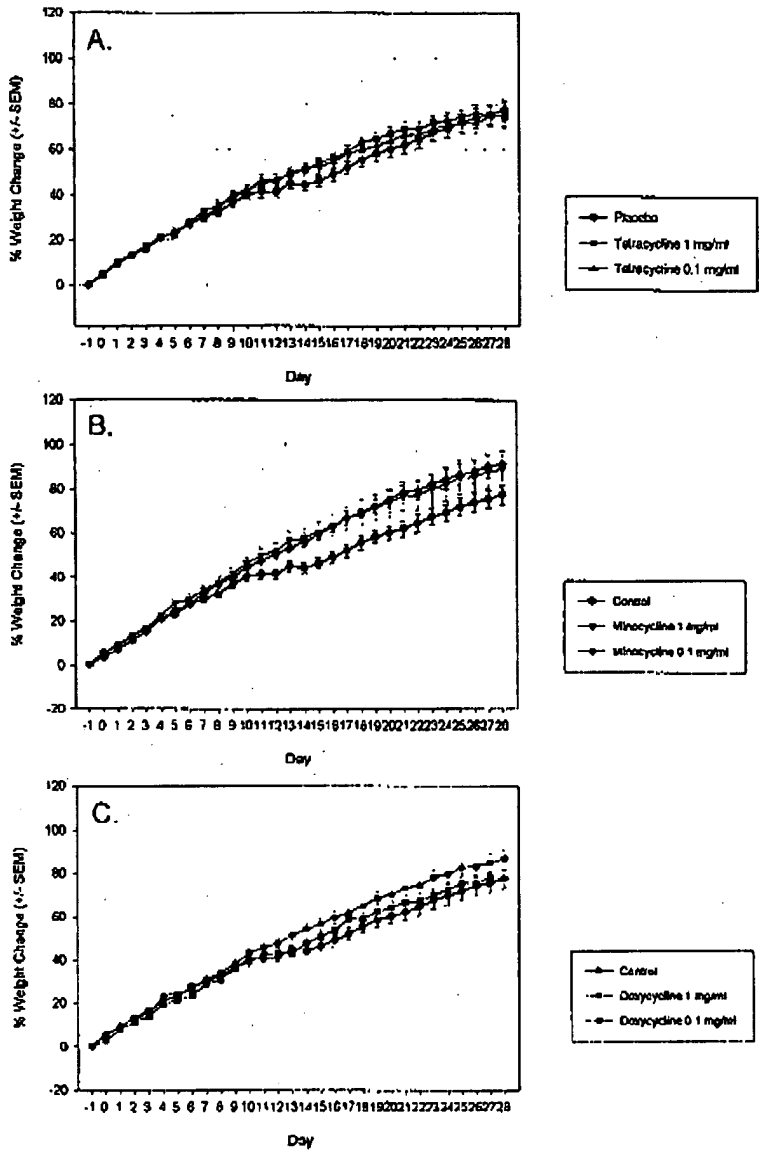


Figure 13. ORA-03. Percent weight change. Animals were weighed daily and group means and standard errors of the mean (SEM) calculated for each day. A. Compares weight gain trends for the groups receiving tetracycline at 1 mg/ml and 0.1 mg/ml with the placebo group. B. Compares the placebo group with groups receiving minocycline at 1 mg/ml and 0.1 mg/ml. C. Compares the placebo group with groups receiving doxycycline at 1 mg/ml and 0.1 mg/ml.

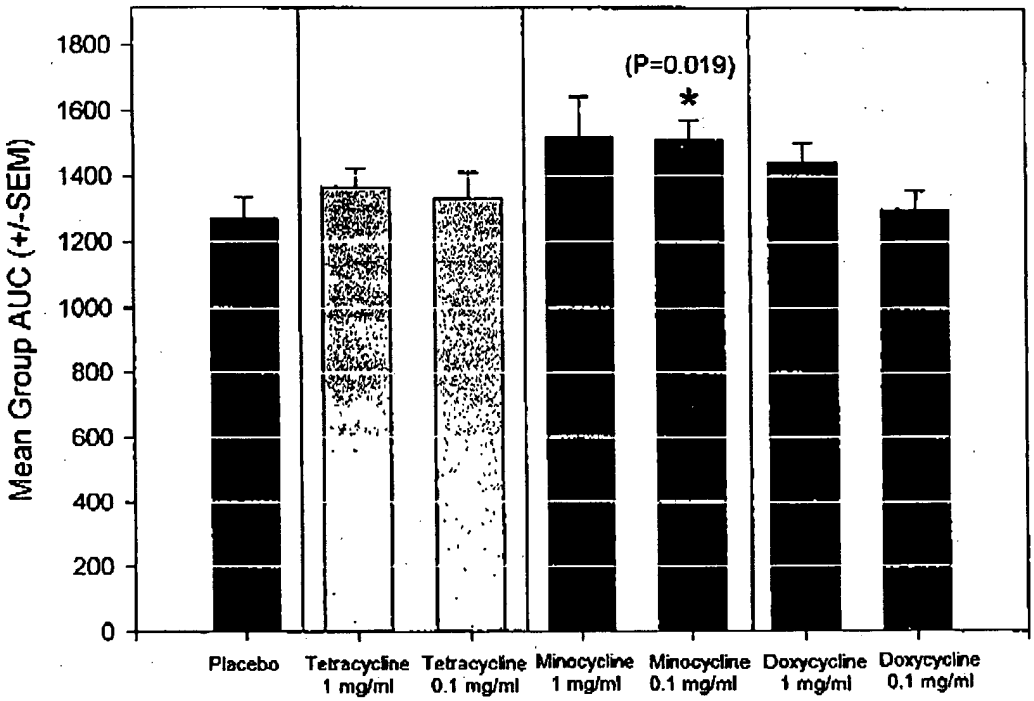


Figure 14. ORA-03. The area under the curve (AUC) was calculated for the percent weight change exhibited by each animal in the study. This calculation was made using the trapezoidal rule transformation. Group means were calculated and are shown with error bars representing SEM for each group. An unpaired t-test was done to compare these groups. The 0.1 mg/ml minocycline group was the only group that showed significantly greater weight gain than the placebo group.

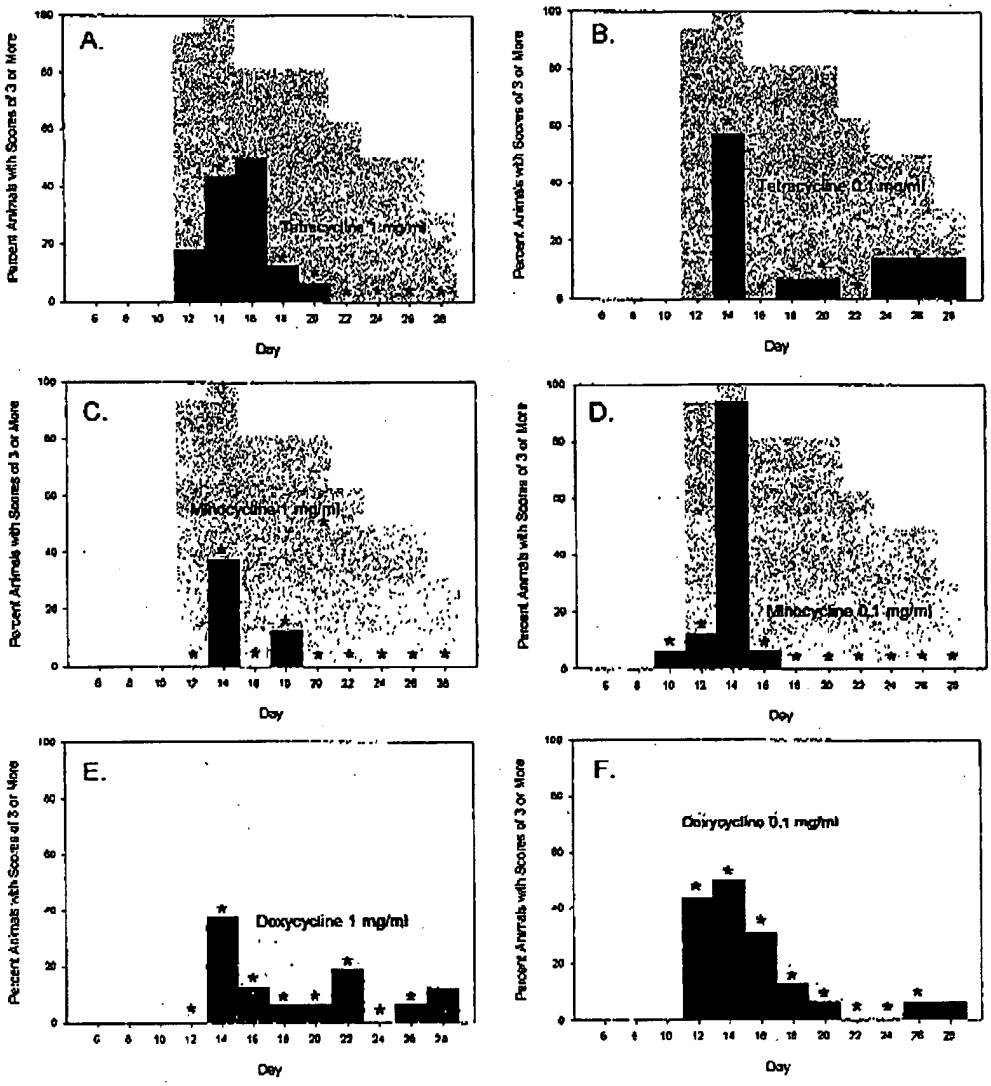


Figure 15. ORA-03. Daily mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the percentage of animals with a score of 3 or more was determined daily for each group. The colored plot represents the drug-treated group while the gray plot represents the placebo group. Statistical significance of the daily observed differences was calculated using chi-square analysis, significant differences between placebo and treated groups are indicated by asterisks. Treatment with tetracycline at 1 mg/ml (A) and 0.1 mg/ml (B) had strong significant efficacy in treating mucositis. Minocycline treatment resulted in a large, highly significant reduction in ulcerative mucositis at both 1 mg/ml (C) and 0.1 mg/ml (D). Doxycycline treatment, in a manner similar to the other tetracyclines, had strong significant efficacy in treating mucositis at 1 mg/ml (E) and 0.1 mg/ml.

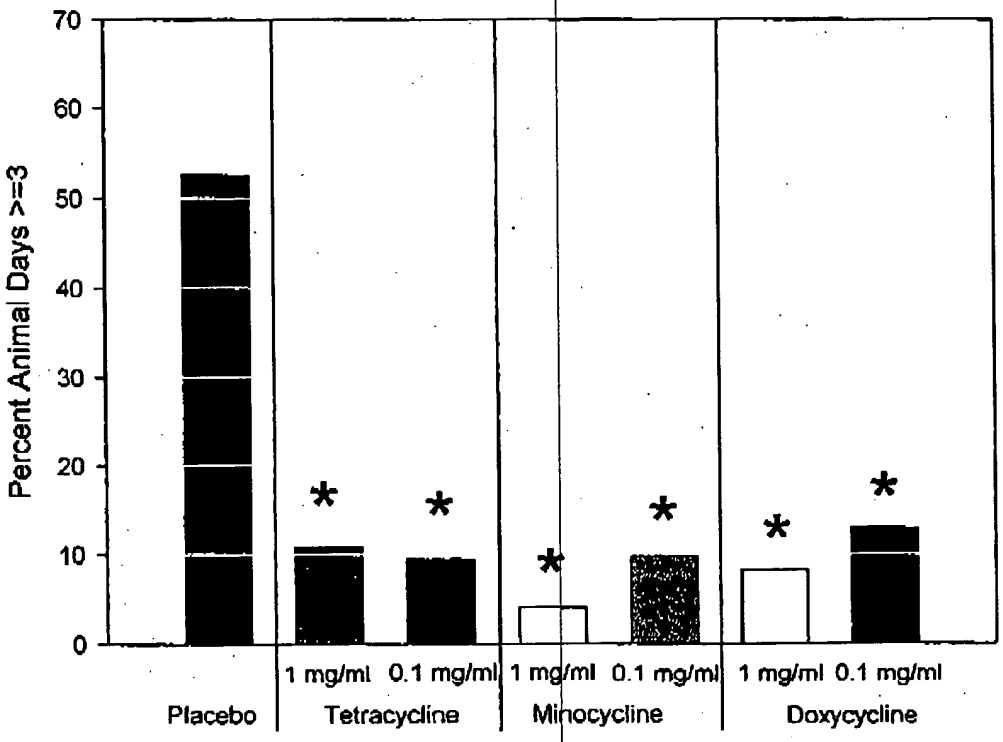


Figure 16. ORA-03. Animal days with mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the total number of days in which an animal exhibited an elevated score were summed and expressed as a percentage of the total number of days. Statistical significance of observed differences was calculated using chi-square analysis. Asterisks indicate significant differences between individual treatment groups and placebo animals. All six tetracycline-treated groups had a significantly lower percentage of days with ulcers.

ORA-03 Chi Square Analysis of Daily Mucositis Scores >=3 (P Values)
Significant Scores in Red

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Placebo v 1 mg/ml Tetracycline	1.000	1.000	1.000	<0.001	0.109	0.121	<0.001	<0.001	<0.001	0.002	0.002	0.043
Placebo v 0.1 mg/ml Tetracycline	1.000	1.000	1.000	<0.001	0.015	<0.001	<0.001	<0.001	<0.001	0.058	0.058	0.399
Placebo v I mg/ml Minocycline	1.000	1.000	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.002	0.043
Placebo v 0.1 mg/ml Minocycline	1.000	1.000	1.000	0.191	1.000	<0.001	<0.001	<0.001	<0.001	0.002	0.002	0.043
Placebo v 1 mg/ml Doxycycline	1.000	1.000	1.000	0.041	<0.001	<0.001	<0.001	<0.001	0.031	0.002	0.015	0.394
Placebo v 0.1 mg/ml Doxycycline	1.000	1.000	1.000	0.068	0.102	0.013	<0.001	<0.001	<0.001	0.002	0.015	0.172

Table 6. Chi-square analysis of the distribution of daily scores. The percent distrubution of scores of 3 or more compared to scores less than 3 is shown in Figure 15. The significance of differences observed in that analysis is determined by Chi-square analysis. Values shown here are P values for each calculation. Significance is defined as a P value less than or equal to 0.050.

ORA-03 Mann Whitney Rank Sum Test of Daily Mucositis Scores (P Values)
Significant Scores in Red

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Placebo v 1 mg/ml Tetracycline	0.556	0.298	0.265	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	0.006	<0.001
Placebo v 0.1 mg/ml Tetracycline	0.268	0.317	0.098	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	0.043	0.003
Placebo v 1 mg/ml Minocycline	0.776	0.636	0.508	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Placebo v 0.1 mg/ml Minocycline	0.776	0.439	0.895	<0.001	0.015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Placebo v 1 mg/ml Doxycycline	0.556	0.014	0.663	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.003	<0.001
Placebo v 0.1 mg/ml Doxycycline	0.985	0.231	0.835	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001

Table 7. The significance of group differences differences observed in daily mucositis scores was determined using the Mann-Whitney rank sum test. This nonparametric statistic is appropriate for the visual mucositis scoring scale. The P values for each calculation are shown.

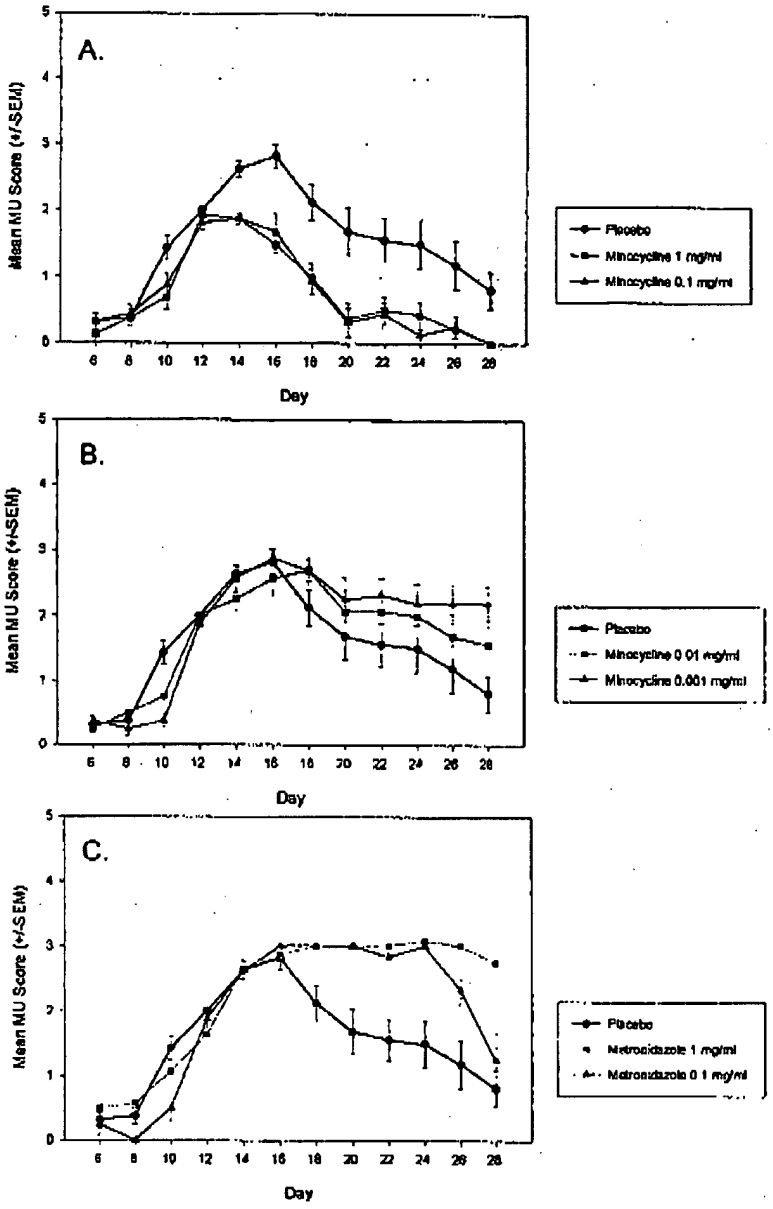


Figure 17. ORA-04. Mean group mucositis scores were obtained for the animal groups receiving topical treatment in water (placebo). Error bars represent the standard error of the mean (SEM). A. Comparison of the placebo group with groups receiving minocycline at either 1 mg/ml or 0.1 mg/ml. B. Comparison of the placebo group with animals receiving minocycline at either 0.01 mg/ml or 0.001 mg/ml. C. Comparison of the placebo group with animals receiving metronidazole at either 1 mg/ml or 0.1 mg/ml. Minocycline has efficacy at the two higher doses (A), but loses efficacy at 0.01 mg/ml (B). Metranidazole, a non-tetracycline antibiotic, appears to worsen oral ulcerative mucositis at both doses studied (C).

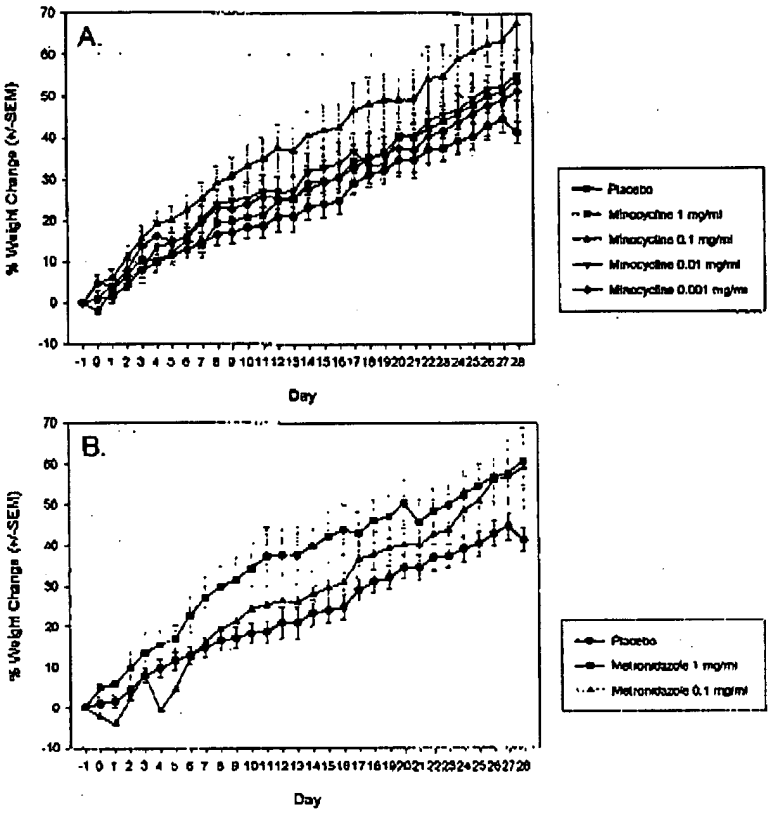


Figure 18. ORA-04. Percent weight change. Animals were weighed daily and group means and standard errors of the mean (SEM) calculated for each day. A. Compares weight gain trends for the groups receiving minocycline at 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml with the placebo group. B. Compares the placebo group with groups receiving metronidazole at 1 mg/ml and 0.1 mg/ml.

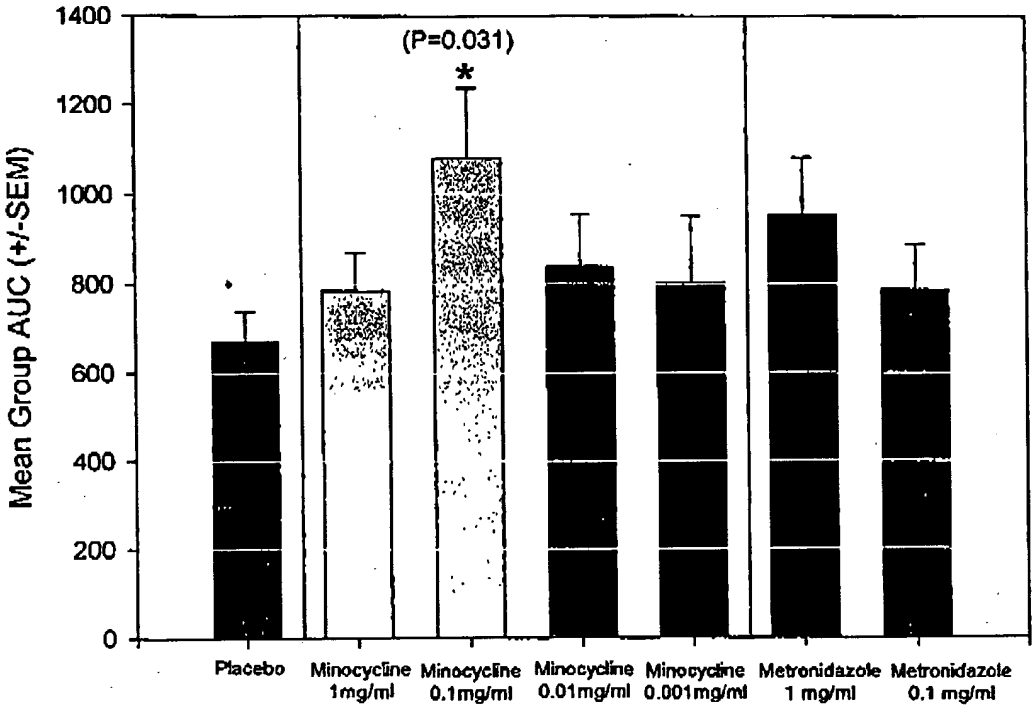


Figure 19. ORA-04. The area under the curve (AUC) was calculated for the percent weight change exhibited by each animal in the study. This calculation was made using the trapezoidal rule transformation. Group means were calculated and are shown with error bars representing SEM for each group. An unpaired t-test was done to compare these groups. The hamsters treated with minocycline at 0.1 mg/ml showed significant weight gain when compared to the placebo group.

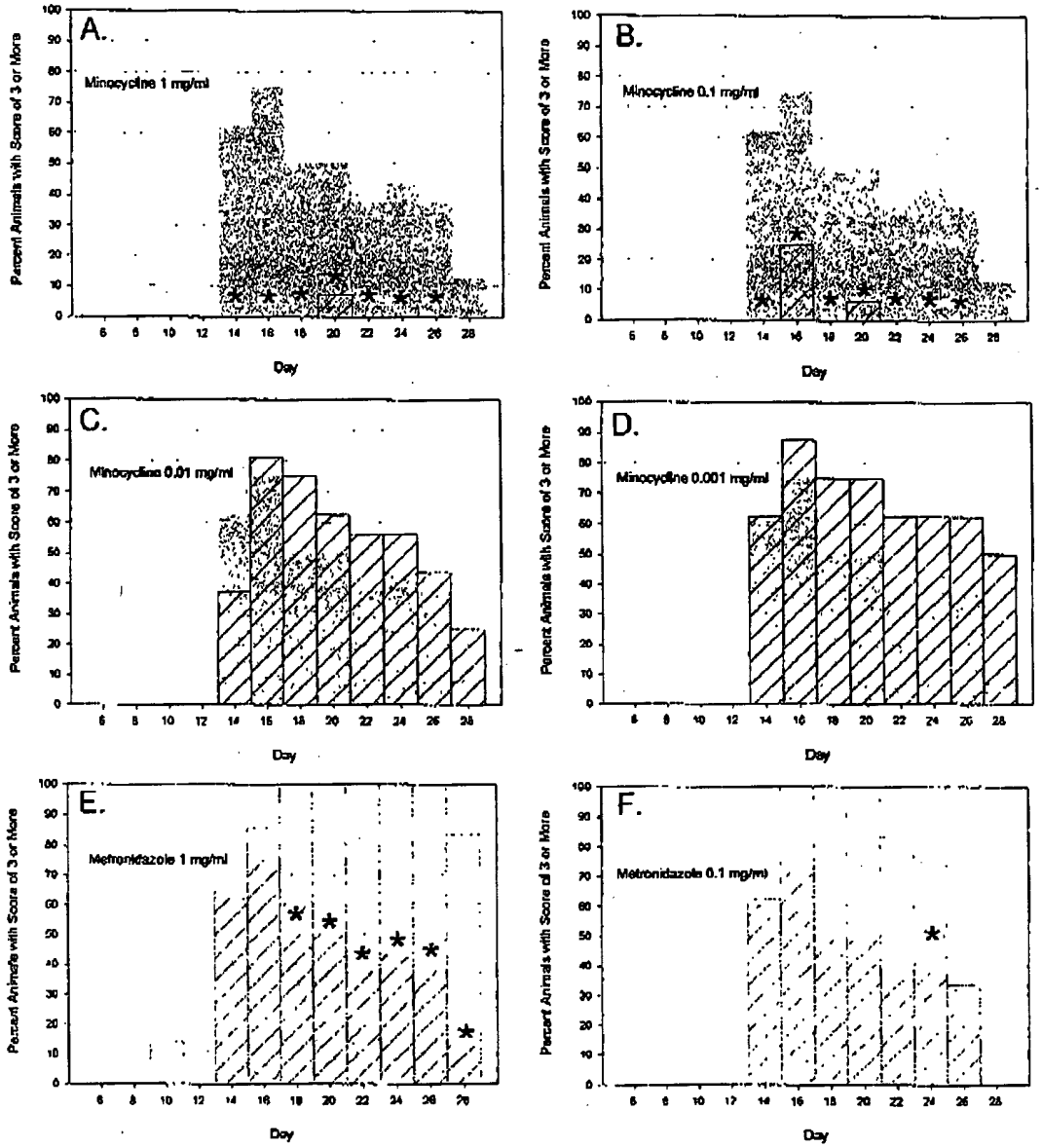


Figure 20. ORA-04. Daily mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), The percentage of animals with a score of 3 or more was determined daily for each group. The colored plot represents the drug-treated group while the gray plot represents the placebo group. Statistical significance of the daily observed differences was calculated using chi-square analysis, significant differences between placebo and treated groups are indicated by asterisks. Treatment with minocycline at 1 mg/ml (A) and 0.1 mg/ml (B) resulted in a large, highly significant reduction in ulcerative mucositis. Minocycline treatment at both 0.01 mg/ml (C) and 0.001 mg/ml (D) had no significant effect on mucositis although mucositis scores were greater (NS) in treated animals when compared to animals in the placebo group. Metronidazole treatment at 1 mg/ml (E) and 0.1 mg/ml (F) both resulted in a significant worsening of mucositis.

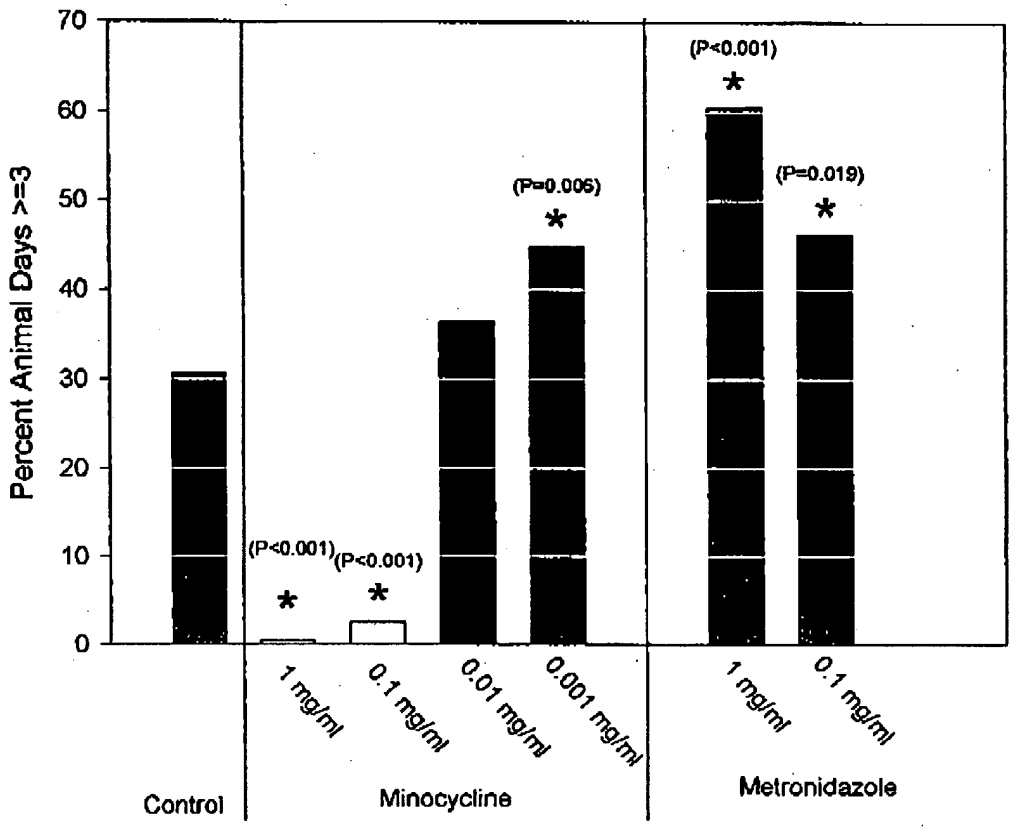


Figure 21. ORA-04. Animal days with mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the total number of days in which an animal exhibited an elevated score were summed and expressed as a percentage of the total number of days. Statistical significance of observed differences was calculated using chi-square analysis. Asterisks indicate significant differences between individual treatment groups and placebo animals. The groups treated with minocycline at 1 mg/ml and 0.1 mg/ml had a significantly lower percentage of days with ulcers. The 0.01 mg/ml dose of minocycline had no significant effect on the mucosal lesions. Hamsters receiving 0.001 mg/ml of minocycline had a significant increase in the percentage of days with scores of 3 or more. Metronidazole had a significant worsening effect on oral mucositis at the doses tested.

ORA-04 Chi Square Analysis of Daily Mucositis Scores >=3 (P Values)
Scores Indicating Significant Improvement over Control Scores are shown in Red
Scores Indicating Significant Worsening Compared with Control Scores are shown in Green

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Control v 1 mg/ml Minocycline	1.000	1.000	1.000	1.000	<0.001	<0.001	0.002	0.015	0.018	0.007	0.018	0.484
Control v 0.1 mg/ml Minocycline	1.000	1.000	1.000	1.000	<0.001	0.013	0.002	0.015	0.018	0.007	0.018	0.484
Control v 0.01 mg/ml Minocycline	1.000	1.000	1.000	1.000	0.289	1.000	0.237	0.722	0.497	0.724	ns	0.654
Control v 0.001 mg/ml Minocycline	1.000	1.000	1.000	1.000	1.000	0.654	0.237	0.237	0.289	0.479	0.289	0.057
Control v 1 mg/ml Metronidazole	1.000	1.000	0.209	1.000	0.781	0.657	0.003	0.003	<0.001	0.003	<0.001	<0.001
Control v 0.1 mg/ml Metronidazole	1.000	1.000	1.000	1.000	1.000	0.262	0.051	0.051	0.149	0.046	1.000	1.000

Table 8. Chi-square analysis of the distribution of daily scores. The percent distrubution of scores of 3 or more compared to scores less than 3 is shown in Figure 20. The significance of differences observed in that analysis is determined by Chi-square analysis. Values shown here are P values for each calculation. Significance is defined as a P value less than or equal to 0.050.

ORA-04 Mann Whitney Rank Sum Test of Daily Mucositis Scores (P Values)
Scores Indicating Significant Improvement over Control Scores are shown in Red
Scores Indicating Significant Worsening Compared with Control Scores are shown in Green

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Control v 1 mg/ml Minocycline	0.372	0.985	0.013	0.774	0.001	<0.001	0.006	0.010	0.032	0.063	0.162	0.042
Control v 0.1 mg/ml Minocycline	0.985	0.777	0.050	0.370	0.004	0.005	0.003	0.006	0.018	0.016	0.092	0.035
Control v 0.01 mg/ml Minocycline	0.776	0.558	0.013	0.985	0.156	0.762	0.156	0.545	0.335	0.835	0.461	0.109
Control v 0.001 mg/ml Minocycline	0.776	0.557	<0.001	0.554	0.985	0.924	0.167	0.335	0.117	0.234	0.064	0.004
Control v 1 mg/ml Metronidazole	0.392	0.631	0.227	0.186	0.950	0.983	0.020	0.020	0.006	0.008	0.005	<0.001
Control v 0.1 mg/ml Metronidazole	0.829	0.147	0.011	0.640	0.975	0.643	0.081	0.081	0.059	0.050	0.196	0.420

Table 9. The significance of group differences differences observed in daily mucositis scores was determined using the Mann-Whitney rank sum test. This nonparametric statistic is appropriate for the visual mucositis scoring scale. The P values for each calculation are shown.

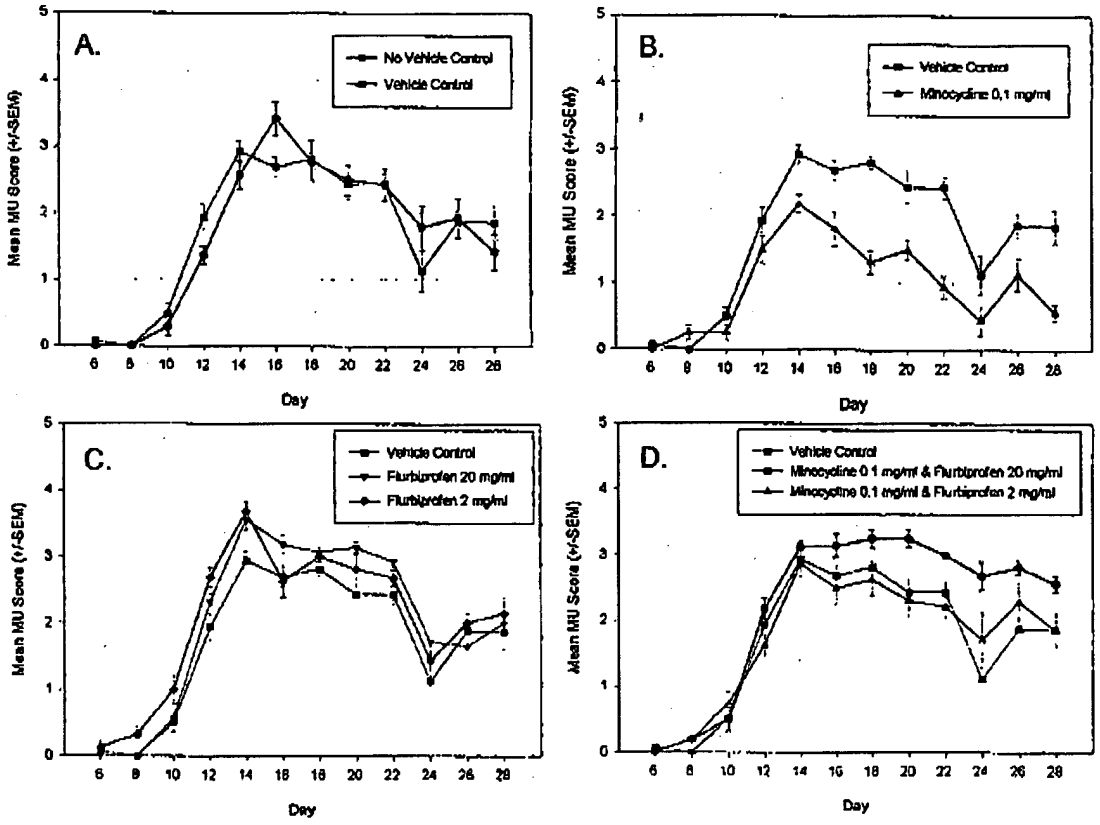


Figure 22. ORA-05. Mean group mucositis scores were obtained for the animal groups receiving topical treatment in water (No vehicle Control) and the animals treated with the cyclodextrin vehicle (Vehicle Control). Error bars represent the standard error of the mean (SEM). A. Comparison of the control groups indicates that the cyclodextrin vehicle has no significant effect on the course of mucositis. B. Comparison of the vehicle control group with animals receiving minocycline at 0.1 mg/ml repeats the observation that 0.1 mg/ml minocycline has significant efficacy in the reduction of the severity of oral; mucositis. C. Comparison of the vehicle control group with animals receiving flurbiprofen at either 20 mg/ml or 2 mg/ml. In this experiment, flurbiprofen , at both doses studied, appears to increase the severity of mucositis. D. Comparison of the vehicle control group with animals receiving a combination of flurbiprofen, at either 20 mg/ml or 2 mg/ml, and minocycline at 0.1 mg/ml. In both combinations, the beneficial effects of minocycline observed in B. are absent. The combination with 2 mg/ml flurbiprofen has no apparent effect on mucositis while the combination with 20 mg/ml flurbiprofen worsens mucositis despite the presence of 0.1 mg/ml minocycline in the combination.

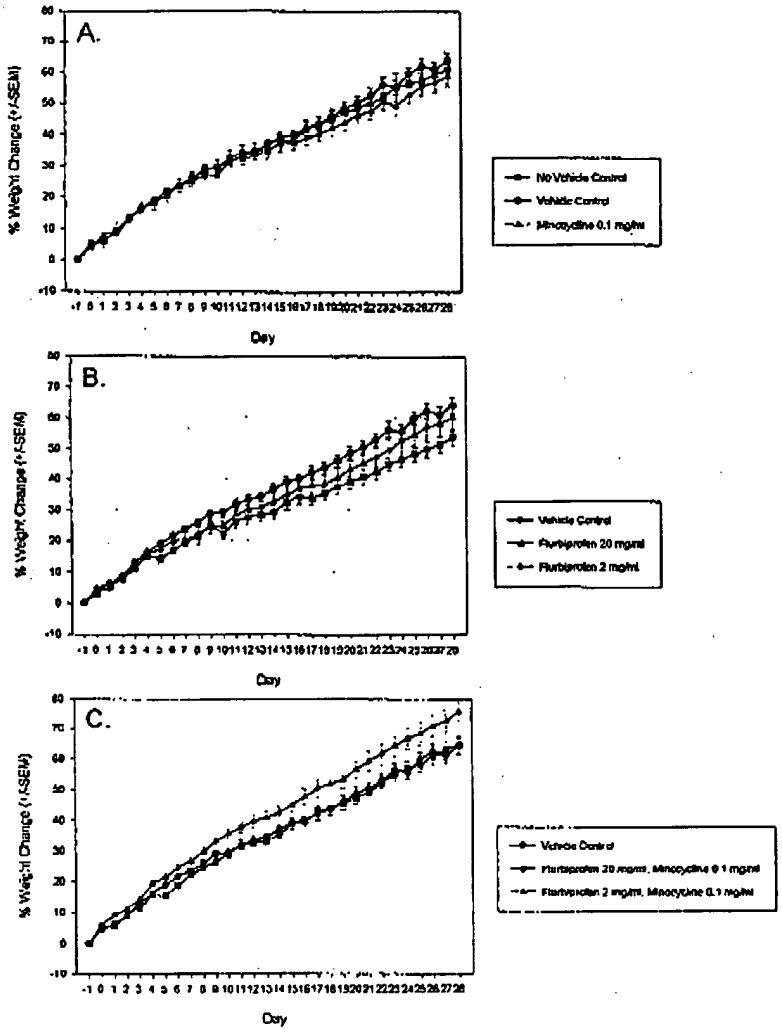


Figure 23. ORA-05. Percent weight change. Animals were weighed daily and group means and standard errors of the mean (SEM) calculated for each day. A. Compares weight gain trends for the control groups and the group receiving minocycline at 0.1 mg/ml. B. Compares the vehicle control group with groups receiving flurbiprofen at 20 mg/ml and 2 mg/ml. C. Compares the vehicle control group with groups receiving a combination of minocycline at 0.1 mg/ml and flurbiprofen at either 20 mg/ml or 2 mg/ml.

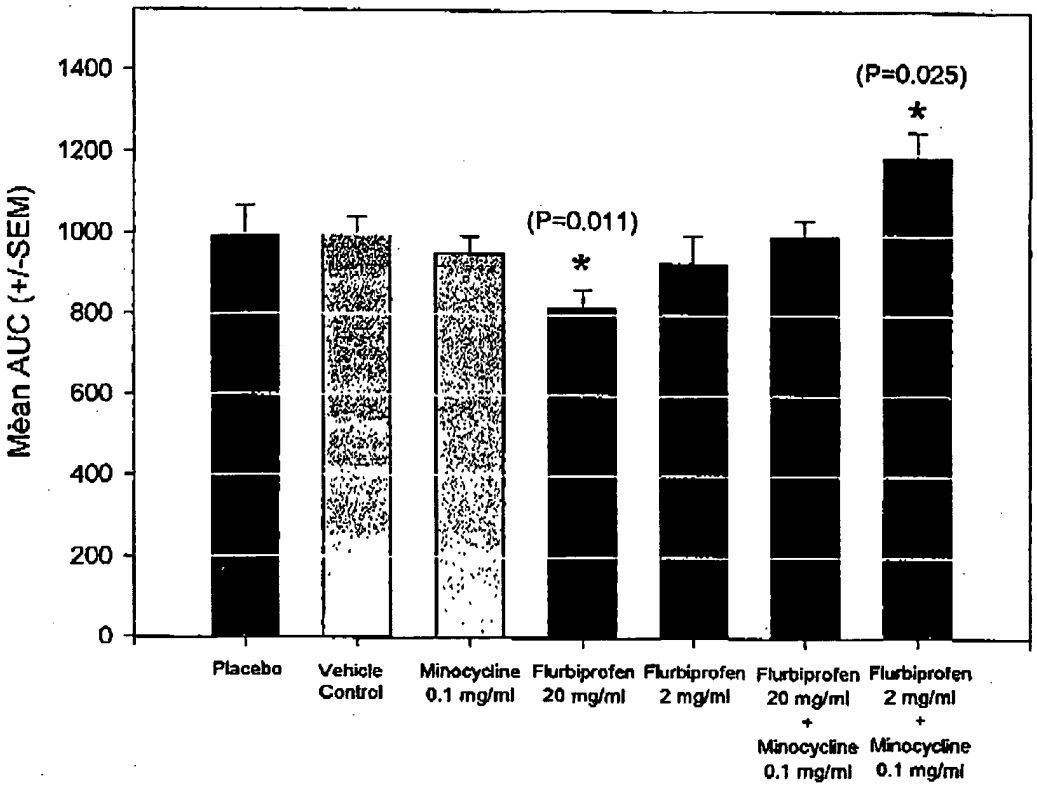


Figure 24. ORA-05. The area under the curve (AUC) was calculated for the percent weight change exhibited by each animal in the study. This calculation was made using the trapezoidal rule transformation. Group means were calculated and are shown with error bars representing SEM for each group. An unpaired t-test was done to compare these groups. The group treated with flurbiprofen at 20 mg/ml lost significantly more weight than the vehicle control group. The group treated with a combination of 0.1 mg/ml of minocycline and 2 mg/ml of flurbiprofen gained significantly more weight than did the vehicle control group.

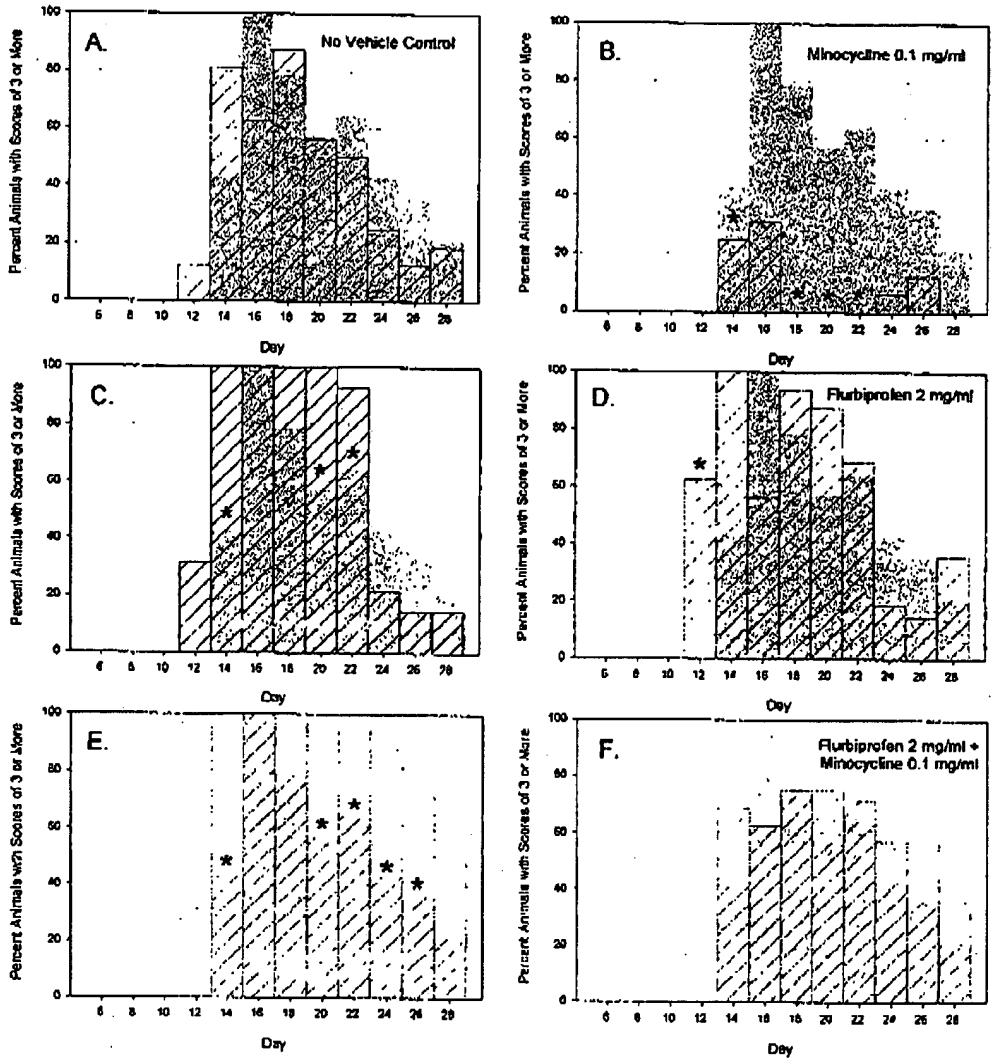


Figure 25. ORA-05. Daily mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), The percentage of animals with a score of 3 or more was determined daily for each group. The colored plot represents the drug-treated group while the gray plot represents the placebo group. Statistical significance of the daily observed differences was calculated using chi-square analysis, significant differences between placebo and treated groups are indicated by asterisks. In all plots, gray bars represent the vehicle control group. The addition of cyclodextrin as a vehicle had no effect on the course of mucositis (A). Minocycline treatment at 0.1 mg/ml had a significant reduction in the severity and duration of mucositis (B). Treatments with flurbiprofen at 20 mg/ml (C) and 2 mg/ml (D) increased the severity of ulcerative mucositis. The combination of 20 mg/ml flurbiprofen and 0.1 mg/ml minocycline (E) significantly increased the severity of mucositis in a manner similar to that of treatment with 20 mg/ml flurbiprofen alone. The combination of 2 mg/ml flurbiprofen and 0.1 mg/ml minocycline (F) had no effect on the course of mucositis.

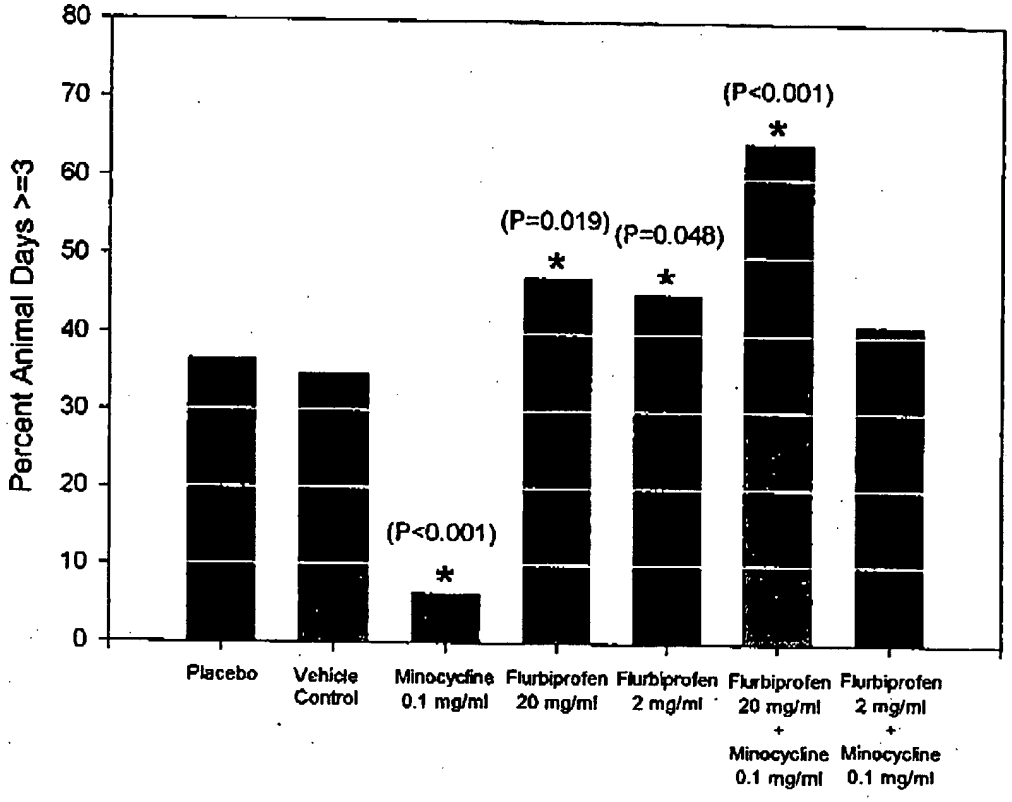


Figure 26. ORA-05. Animal days with mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the total number of days in which an animal exhibited an elevated score were summed and expressed as a percentage of the total number of days. Statistical significance of observed differences was calculated using chi-square analysis. Asterisks indicate significant differences between individual treatment groups and placebo animals. Minocycline treatment significantly reduced the severity of ulcerative mucositis. Treatment with flurbiprofen at both 20 mg/ml and 2 mg/ml significantly increased the severity of mucositis , as did the combination treatment of 20 mg/ml flurbiprofen with 0.1 mg/ml minocycline.

ORA-05 Chi Square Analysis of Daily Mucositis Scores >=3 (P Values)
Significant Efficacy in Red, Significant Worsening in Green

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
NoVehicle Control v Vehicle Control	1.000	1.000	1.000	0.485	0.072	0.019	0.642	0.749	0.676	0.442	0.204	1.000
Vehicle Control v Minocycline 0.1 mg/ml	1.000	1.000	1.000	0.484	0.005	0.156	<0.001	<0.001	0.002	0.333	1.000	0.226
Vehicle Control v Flurbiprofen 20 mg/ml	1.000	1.000	1.000	0.394	0.226	0.018	0.485	0.007	0.017	1.000	1.000	1.000
Vehicle Control v Flurbiprofen 2 mg/ml	1.000	1.000	1.000	0.011	0.226	NS	1.000	0.113	0.472	1.000	1.000	0.417
Vehicle Control v Minocycline 0.1 mg/ml Flurbiprofen 20 mg/ml	1.000	1.000	1.000	0.394	0.226	0.018	0.484	0.007	0.002	0.001	<0.001	0.068
Vehicle Control v Minocycline 0.1 mg/ml Flurbiprofen 2 mg/ml	1.000	1.000	1.000	0.484	0.685	0.715	0.654	0.457	0.411	0.156	0.019	0.417

Table 10. Chi-square analysis of the distribution of daily scores. The percent distrubution of scores of 3 or more compared to scores less than 3 is shown in Figure 25. The significance of differences observed in that analysis is determined by Chi-square analysis. Values shown here are P values for each calculation. Significance is defined as a P value less than or equal to 0.050.

ORA-05 Mann Whitney Rank Sum Test of Daily Mucositis Scores (P Values)
Significant Efficacy in Red, Significant Worsening in Green

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
NoVehicle Control v Vehicle Control	0.784	0.983	0.326	0.043	0.139	0.043	0.587	0.933	0.692	0.190	0.677	0.260
Vehicle Control v Minocycline 0.1 mg/ml	0.774	0.230	0.233	0.345	0.004	0.023	<0.001	0.003	<0.001	0.073	0.018	<0.001
Vehicle Control v Flurbiprofen 20 mg/ml	0.774	0.985	0.895	0.104	0.022	0.058	0.257	0.057	0.045	0.151	0.404	0.765
Vehicle Control v Flurbiprofen 2 mg/ml	0.775	0.133	0.136	0.007	0.005	0.910	0.403	0.225	0.325	0.485	0.786	0.420
Vehicle Control v Minocycline 0.1 mg/ml Flurbiprofen 20 mg/ml	0.774	0.370	0.777	0.273	0.436	0.117	0.102	0.032	0.016	0.003	<0.001	0.045
Vehicle Control v Minocycline 0.1 mg/ml Flurbiprofen 2 mg/ml	0.774	0.370	0.374	0.438	0.636	0.835	0.879	0.970	0.739	0.466	0.118	0.945

Table 11. The significance of group differences differences observed in daily mucositis scores was determined using the Mann-Whitney rank sum test. This nonparametric statistic is appropriate for the visual mucositis scoring scale. The P values for each calculation are shown.

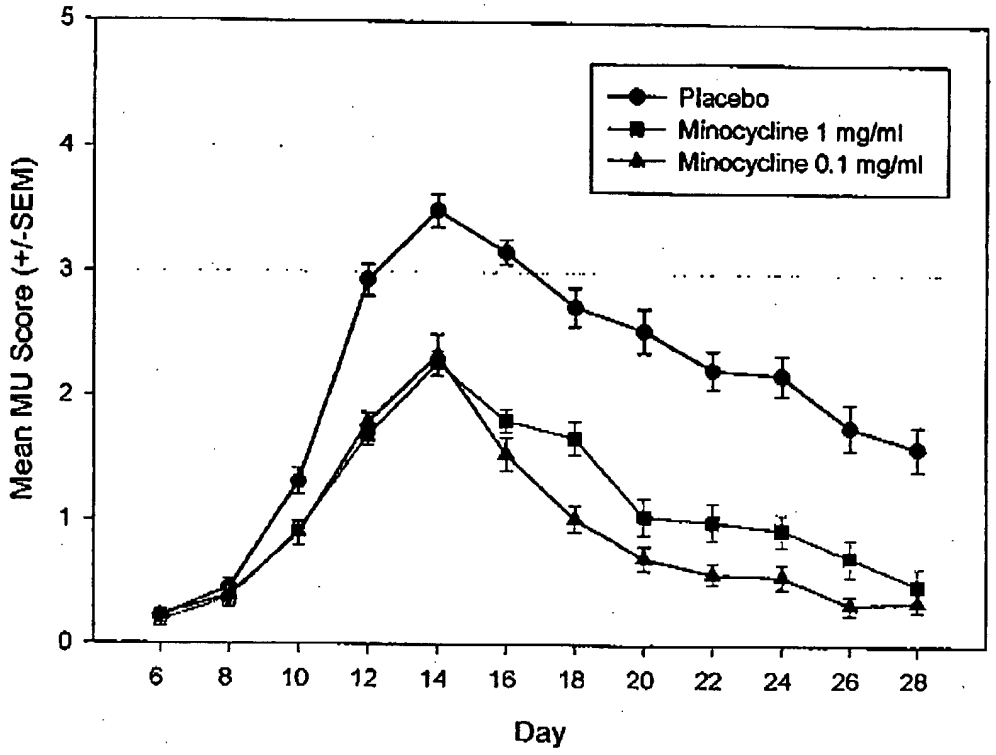


Figure 27. Minocycline metanalysis. Three experimental groups, placebo, minocycline 1 mg/ml and minocycline 0.1 mg/ml, were carried out using identical protocols during the course of this project. The mucositis scores for these groups from ORA-01, ORA-03 and ORA-04 were combined and analyzed. Mean group mucositis scores were obtained as described previously. Error bars represent the standard error of the mean (SEM). A. Comparison of the placebo group with groups receiving minocycline at 1 mg/ml shows a dramatic reduction in the severity of oral mucositis. The reduction in mucositis severity is also observed when minocycline is used at a dose of 0.1 mg/ml. Based on rank sum analysis, statistical differences ($p<0.01$) were noted between both treated groups and the placebo group from day 10 until day 28 (Table 13).

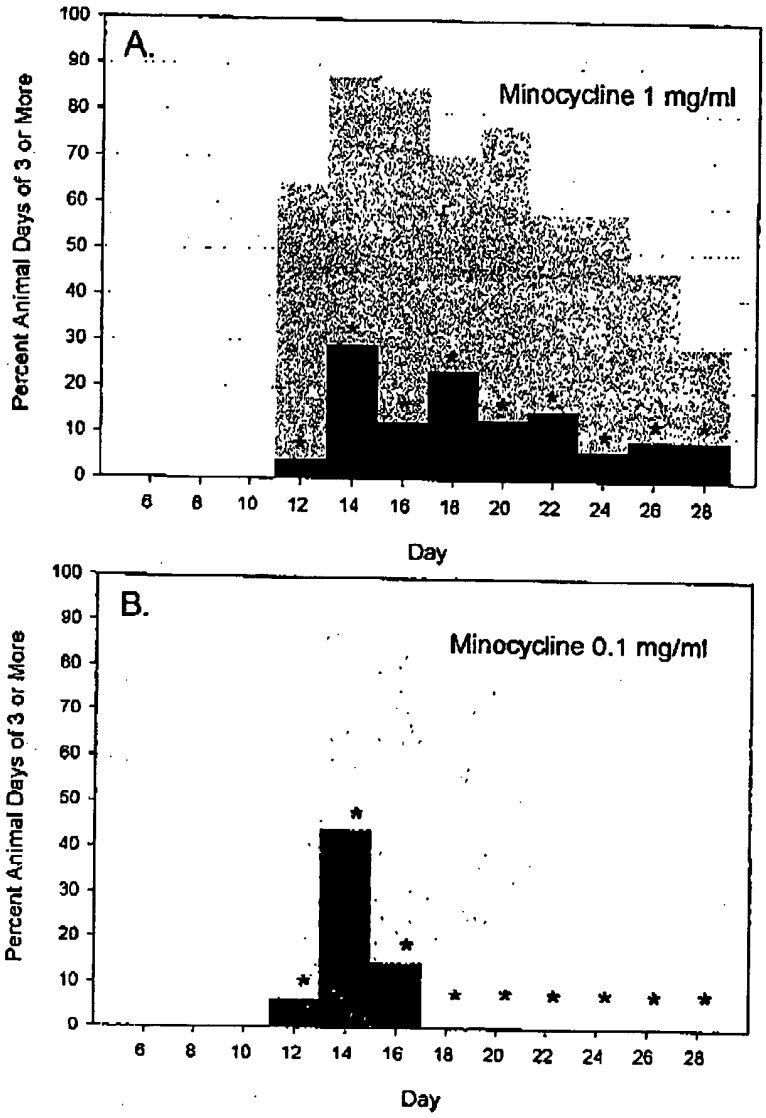


Figure 28. Minocycline metanalysis. Daily mucositis scores of 3 or greater were summed for the placebo, minocycline 1 mg/ml and minocycline 0.1 mg/ml groups from studies ORA-01, ORA-03 and ORA-04. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the percentage of animals with a score of 3 or more was determined daily for each group. The colored plot represents the drug-treated group while the gray plot represents the placebo group. Statistical significance of the daily observed differences was calculated using chi-square analysis, significant differences between placebo and treated groups are indicated by asterisks. Treatment with minocycline at 1 mg/ml (A) and 0.1 mg/ml (B) resulted in a large, highly significant reduction in ulcerative mucositis.

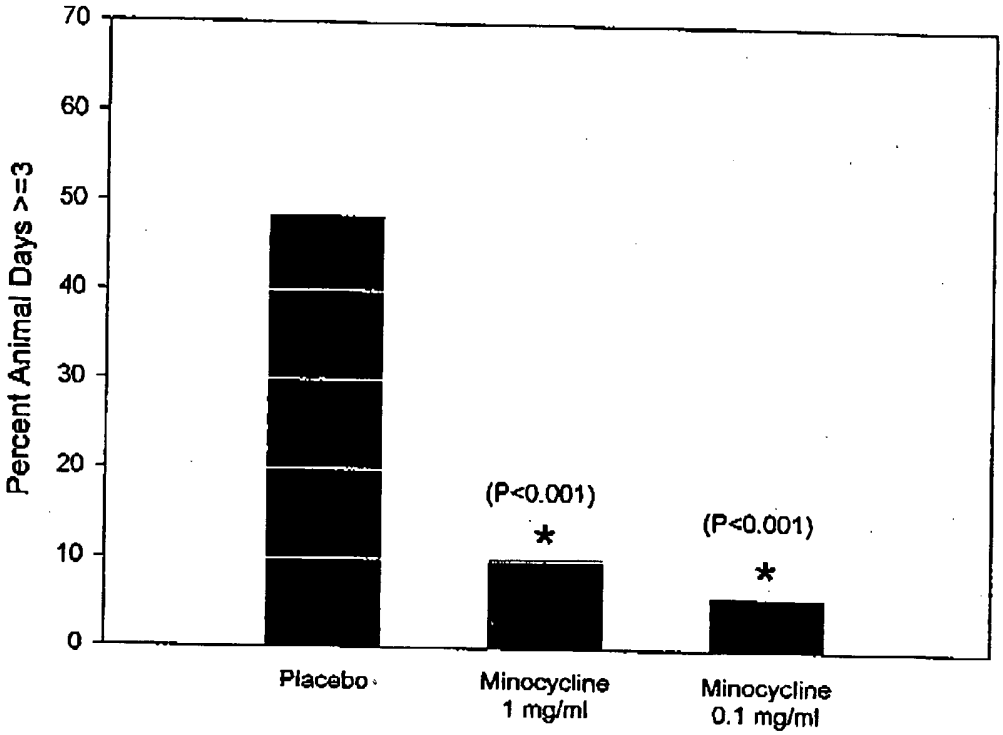


Figure 29. Minocycline Metanalysis. Animal days with mucositis scores of 3 or greater. To examine the levels of clinically significant mucositis, as defined by presentation with open ulcers (a score of 3 or greater), the total number of days in which an animal exhibited an elevated score were summed form ORA-01, ORA-03 and ORA-04 and expressed as a percentage of the total number of days. Statistical significance of observed differences was calculated using chi-square analysis. Asterisks indicate significant differences between individual treatment groups and placebo animals. Both minocycline-treated groups had a significantly lower percentage of days with ulcers.

Minocycline Metanalysis Chi Square Analysis of Daily Mucositis Scores >=3 (P Values)
Significant Scores in Red

	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Control v 1 mg/ml Minocycline	1.000	1.000	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.024
Control v 0.1 mg/ml Minocycline	1.000	1.000	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

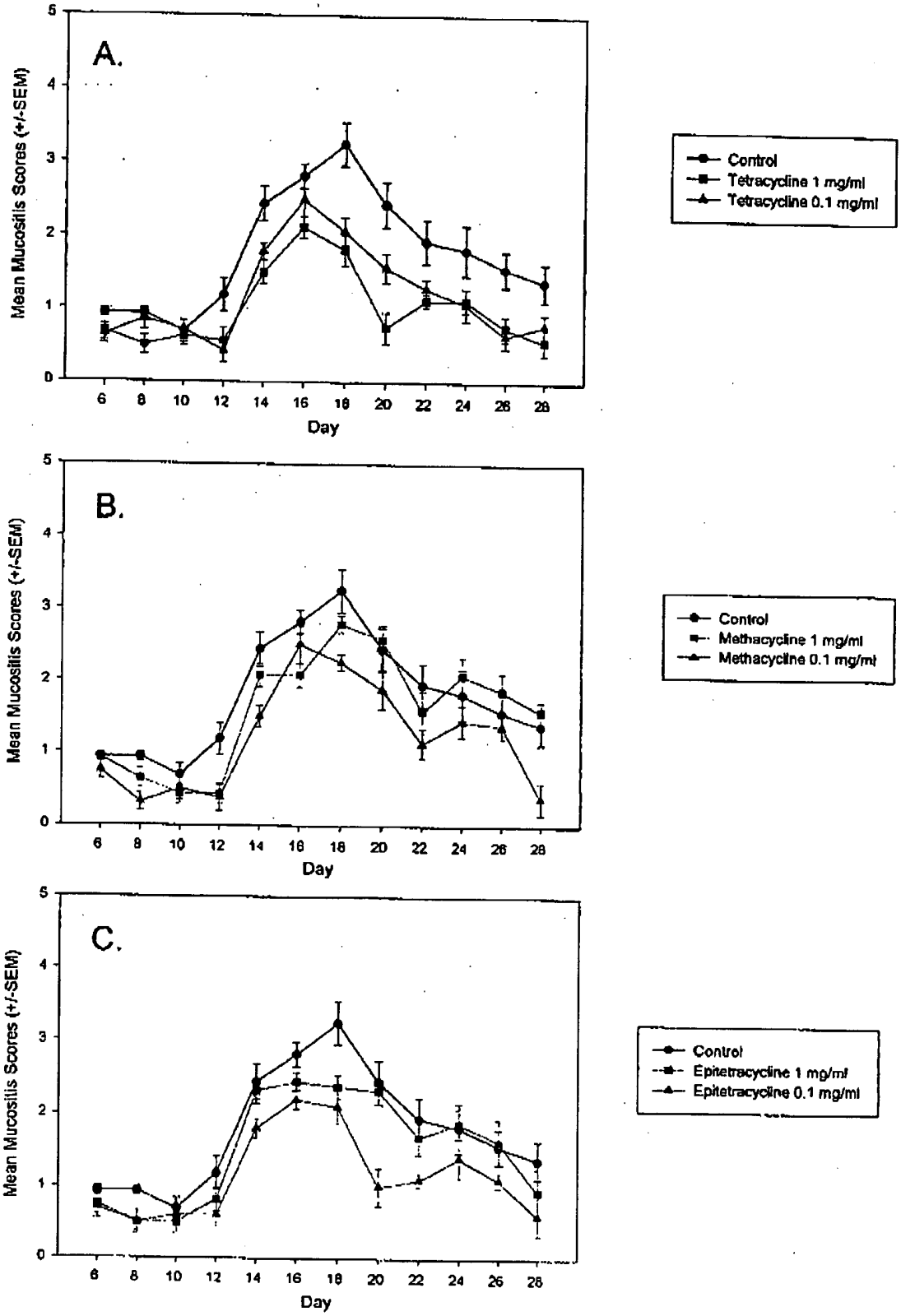
Table 12. Chi-square analysis of the distribution of daily scores. The percent distrubution of scores of 3 or more compared to scores less than 3 is shown in Figure 28. The significance of differences observed in that analysis is determined by Chi-square analysis. Values shown here are P values for each calculation. Significance is defined as a P value less than or equal to 0.050.

Minocycline Metanalysis Mann Whitney Rank Sum Test of Daily Mucositis Scores (P Values)
Significant Scores in Red

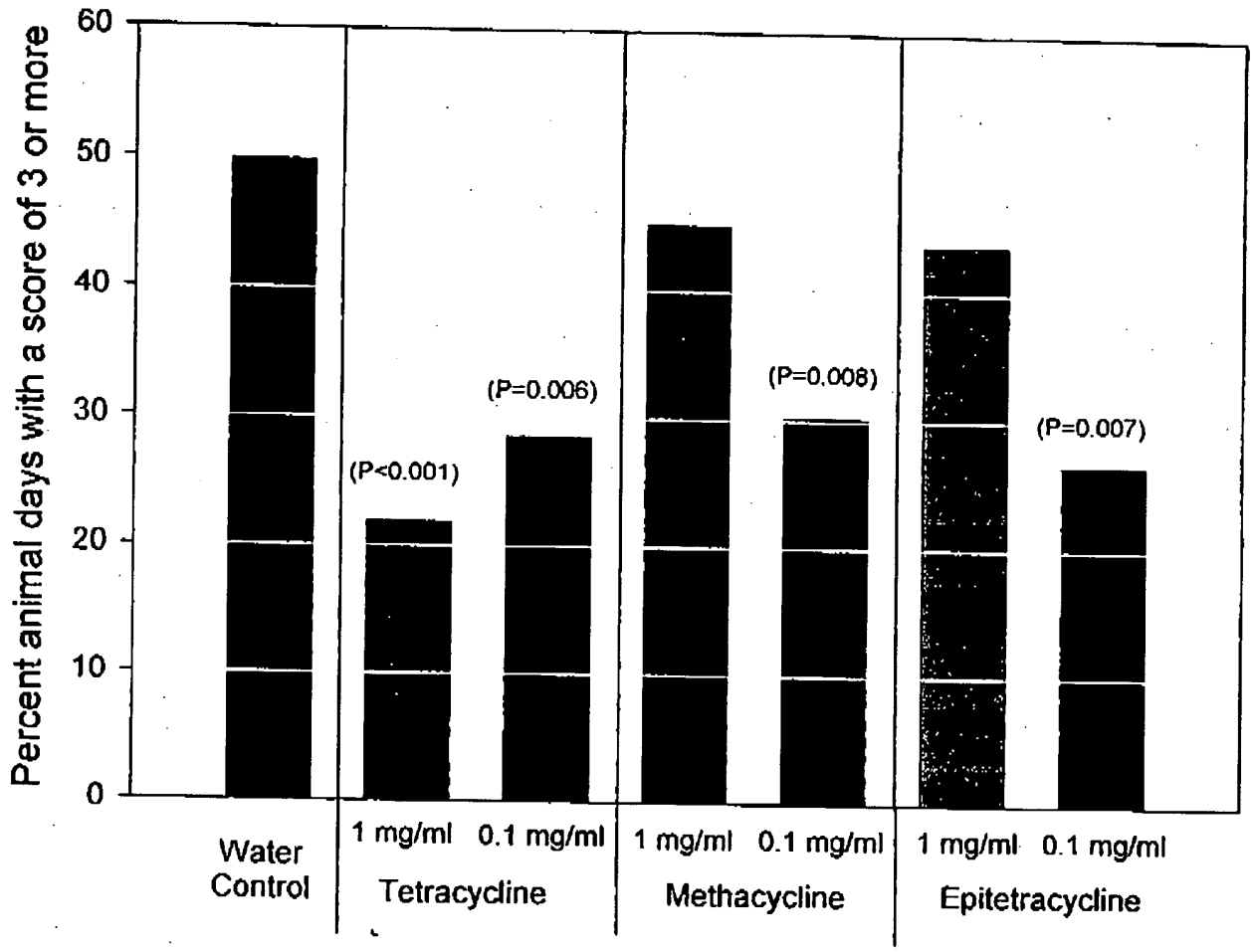
	Day											
GroupComparison	6	8	10	12	14	16	18	20	22	24	26	28
Control v 1 mg/ml Minocycline	0.727	0.555	0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Control v 0.1 mg/ml Minocycline	0.997	0.424	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 13. The significance of group differences differences observed in daily mucositis scores was determined using the Mann-Whitney rank sum test. This nonparametric statistic is appropriate for the visual mucositis scoring scale. The P values for each calculation are shown.

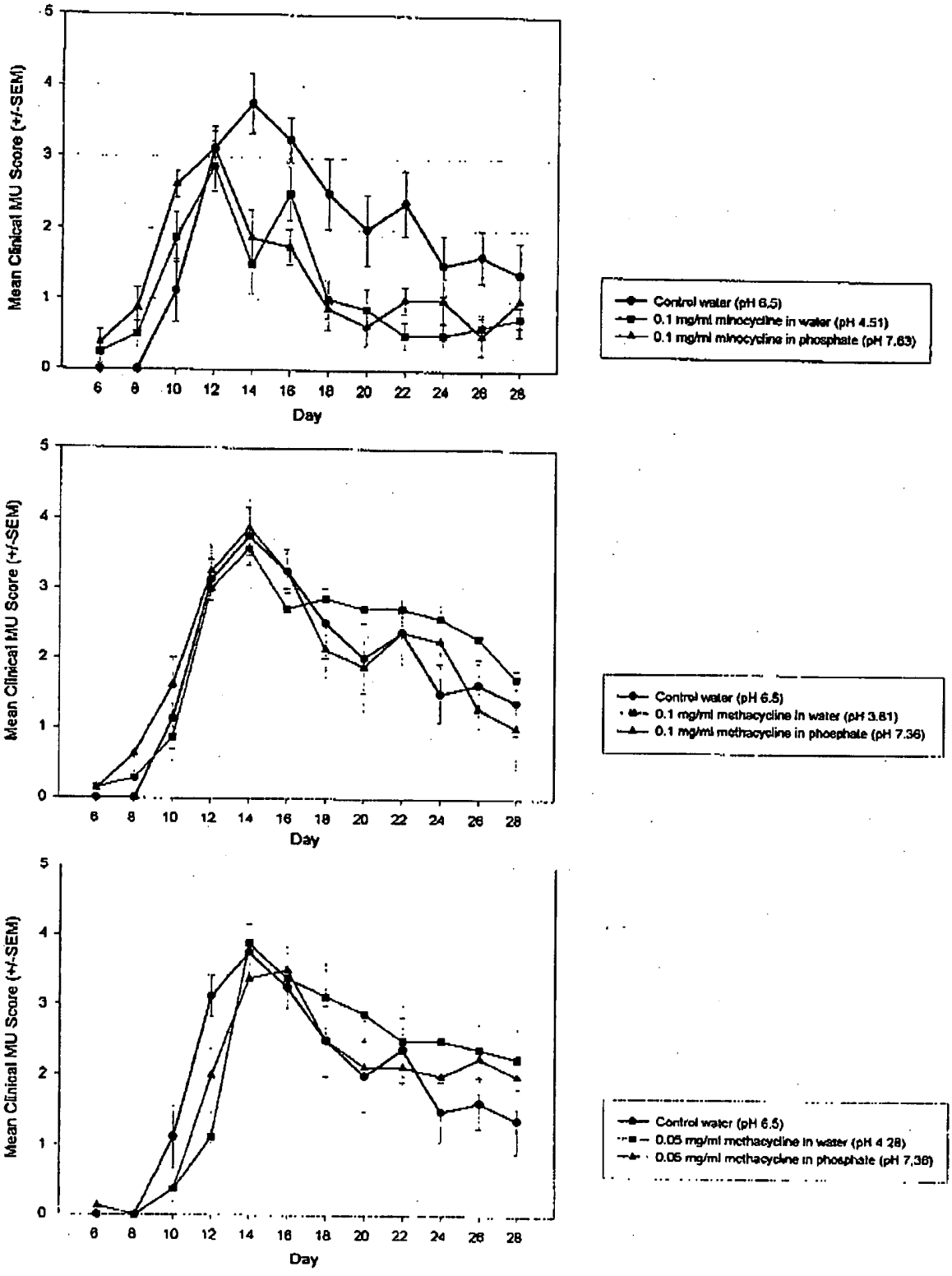
ORA-06 Mucositis Scores



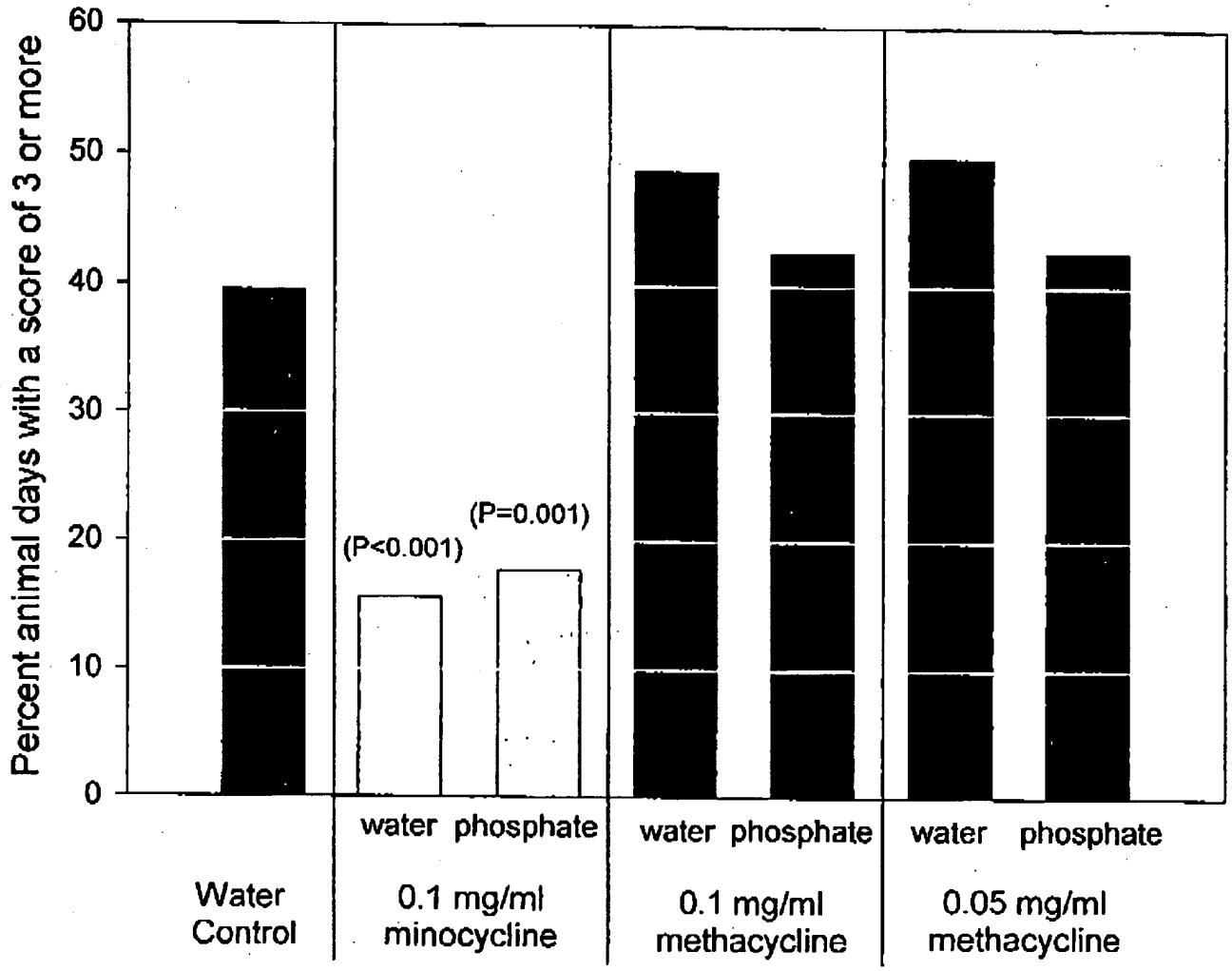
ORA-06 The percentage of the total number of days spent with a score of 3 or more.



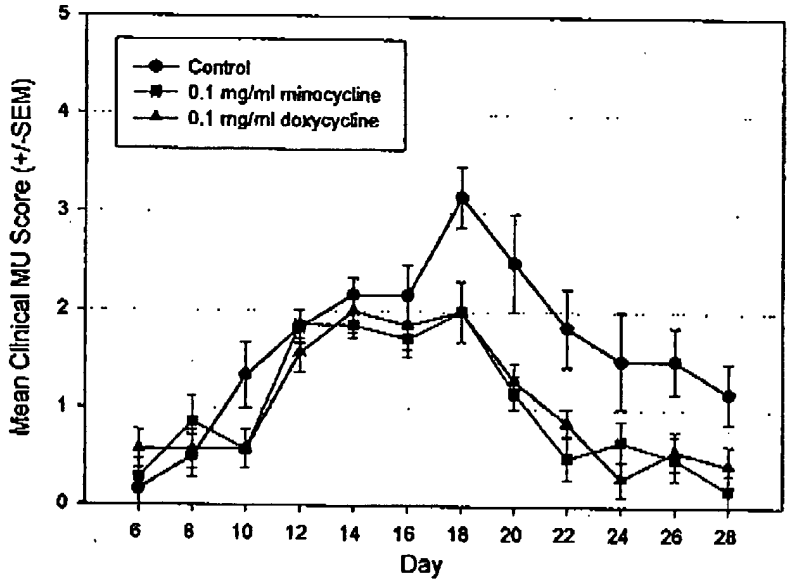
ORA-07 Mucositis scores.

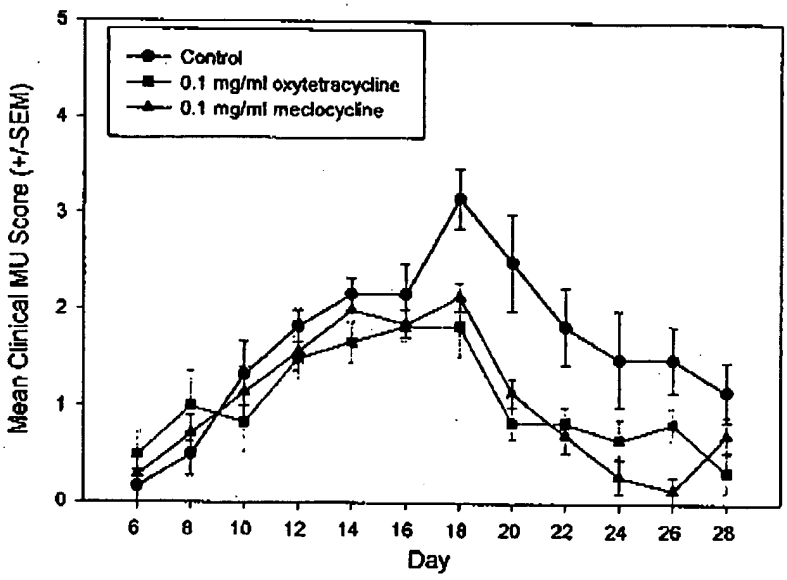


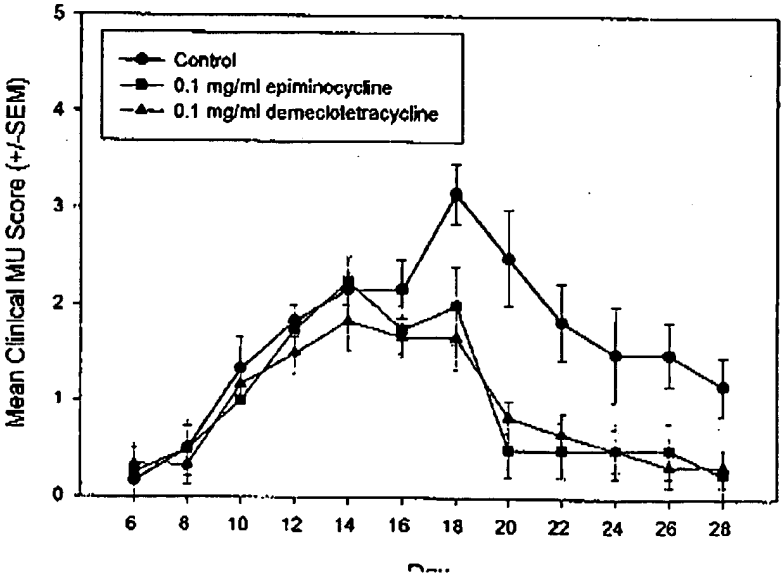
ORA-07 The percentage of the total number of study days spent with a score of 3 or more.



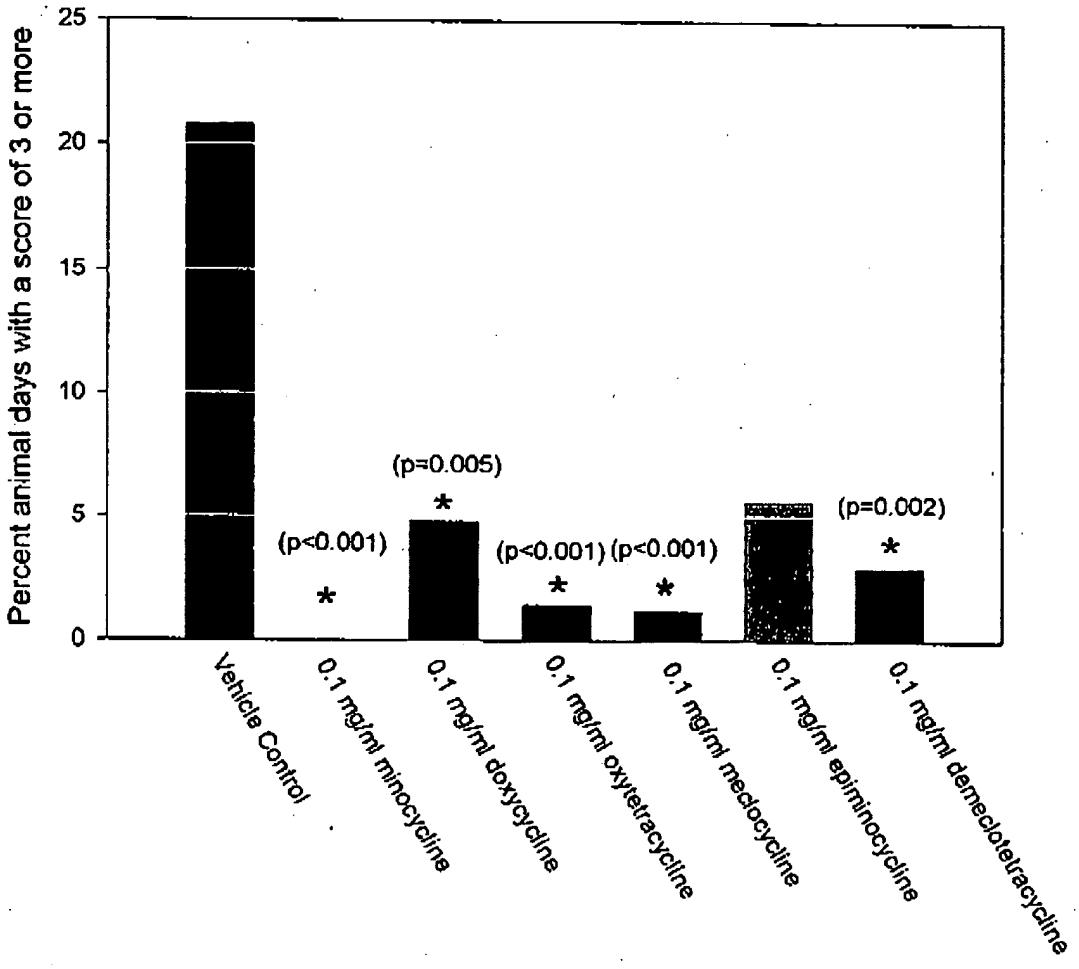
ORA-08 Mucositis Scores



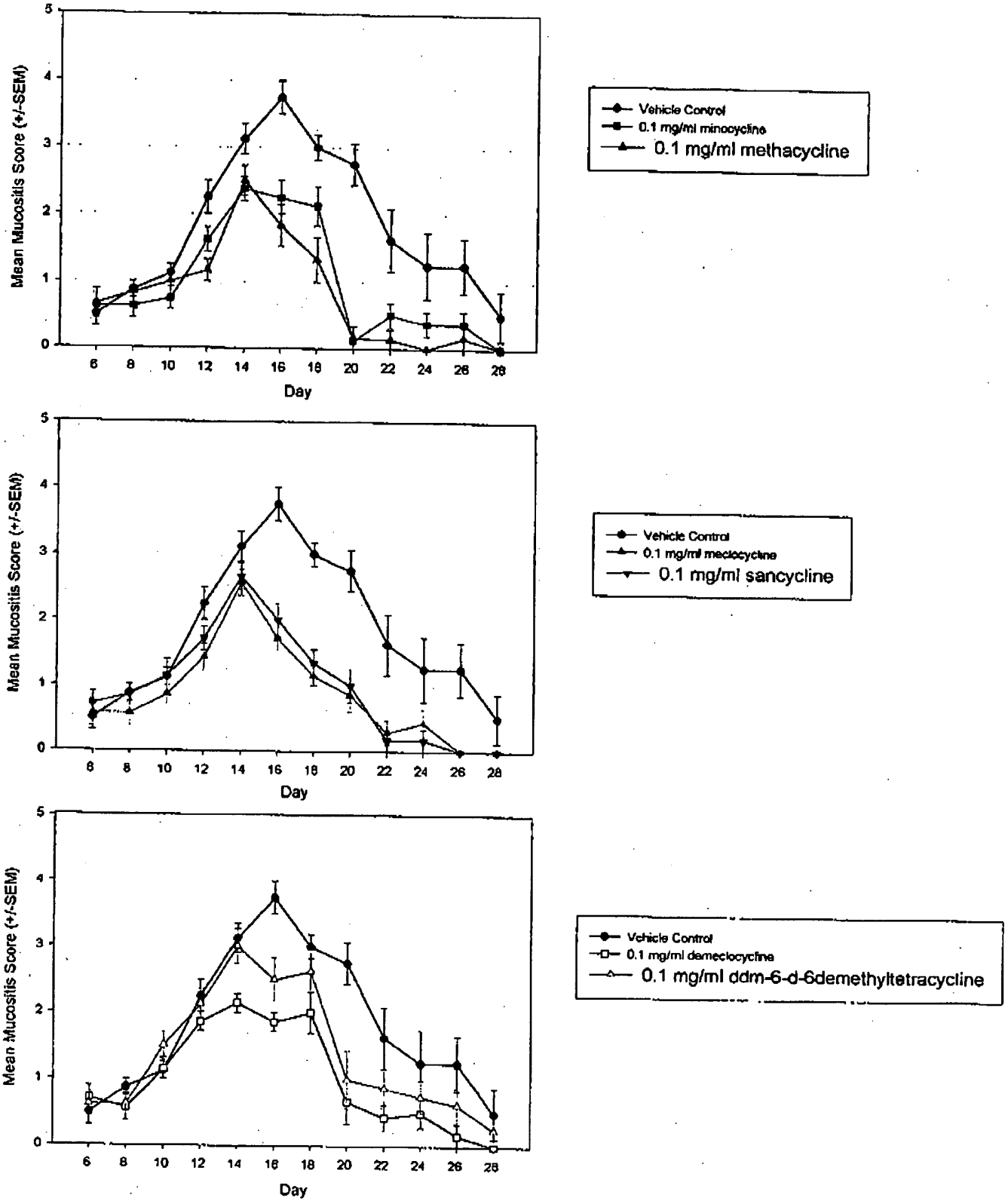




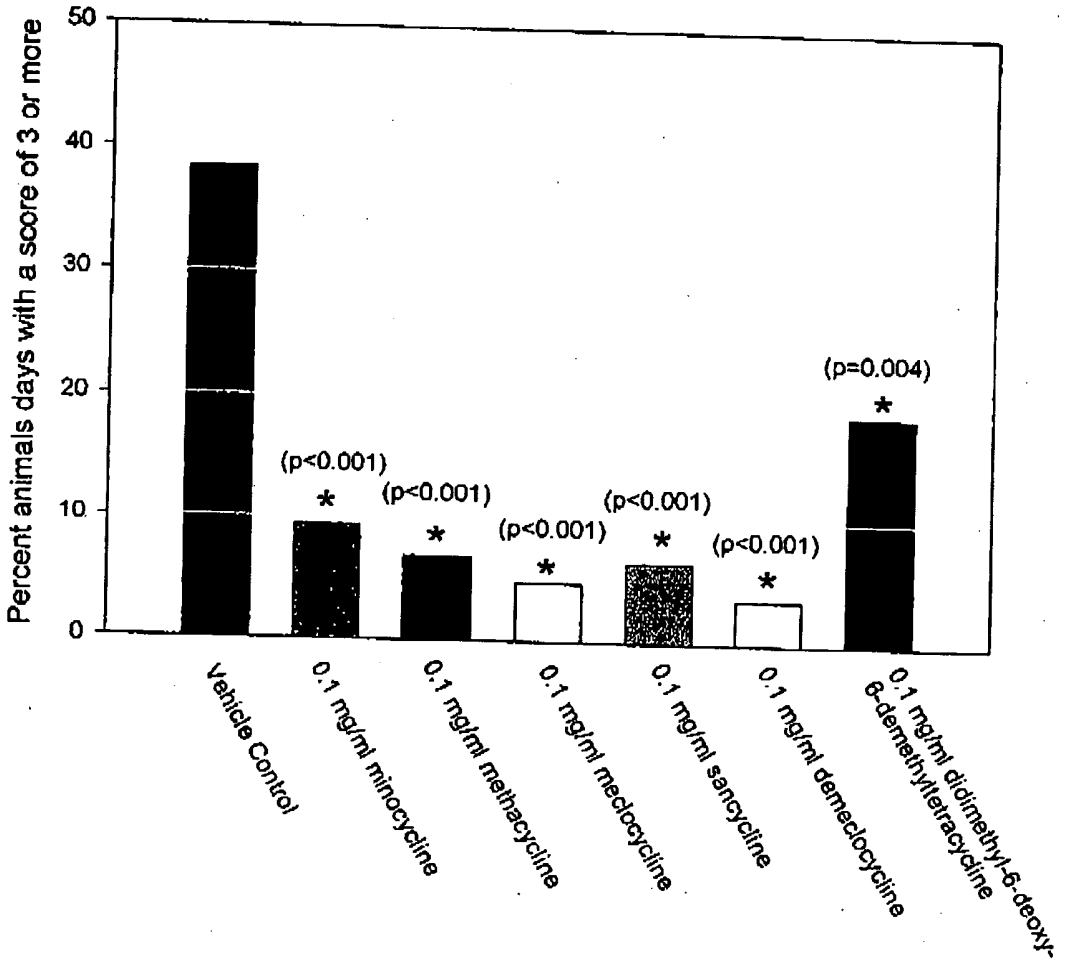
ORA-08 The percentage of the total number of study days spent with a score of 3 or more.



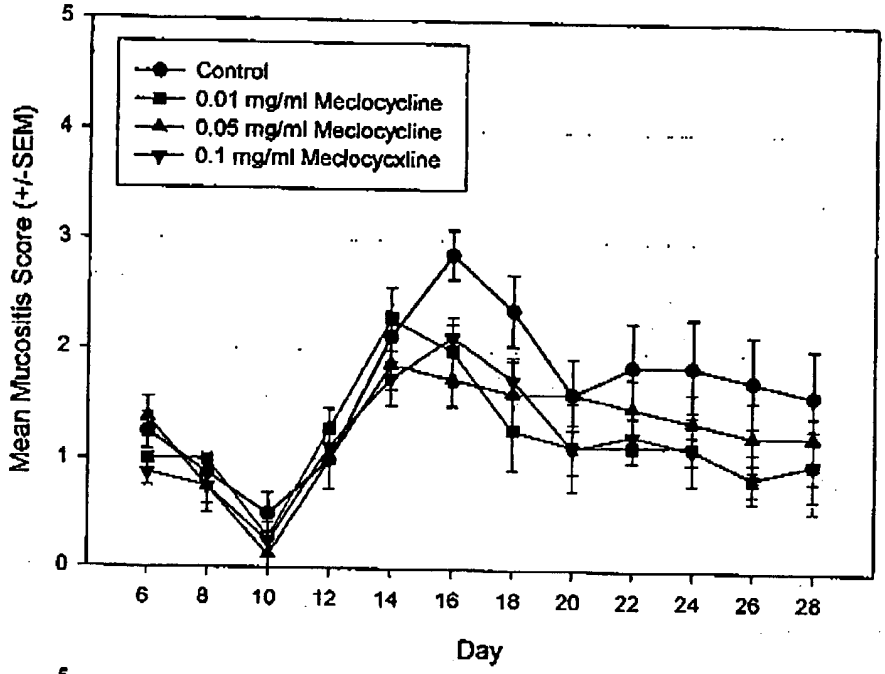
ORA-09 Mucositis Scores

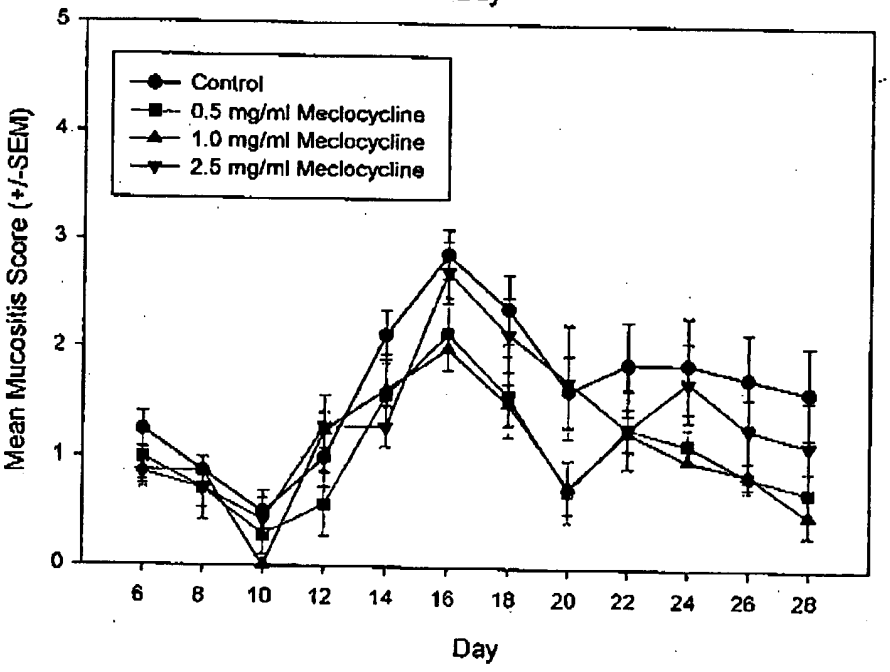


ORA-09 Animal days with a score of 3 or more.



ORA-10 Mucositis Scores





ORA-10 Animals days with a score of 3 or more.